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POPULATION PROBLEMS IN PROTOZOA*

INTRODUCTION

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IT is appropriate that the Symposium on Population Problems in Protozoa, presented jointly by the American Society of Zoologists and the Ecological Society of America, should open with the words of the earliest students in this field. It may be recalled that Leeuwenhoek, the Father of Protozoology, wrote in 1674:

About two hours distant from this Town there lies an inland lake, called the Berkelse Mere, whose bottom in many places is very marshy, or boggy. Its water is in winter very clear, but at the beginning or in the middle of summer it becomes whitish, and there are then little green clouds floating through it; which, according to the saying of the country folk dwelling thereabout, is caused by the dew, which happens to fall at that time, and which they call honey-dew. This water is abounding in fish, which is very good and savoury. Passing just lately over this lake, at a time when the wind blew pretty hard, and seeing the water as above described, I took up a little of it in a glass phial; and examining this water next day, I found floating therein divers earthy particles, and some green streaks, spirally wound serpent-wise, and orderly arranged, after the manner of the copper or tin worms, which distillers use to cool their liquors as they distil over. The whole circumference of each of these streaks was about the thickness of a hair of one's head. Other particles had but the beginning of the foresaid streak; but all consisted of very small green globules joined together: and there were very many small green globules as well. Among these there were, besides, very many little animalcules, whereof some were roundish, while others, a bit bigger, consisted of an oval. On these last I saw two little legs near the

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head, and two little fins at the hindmost end of the body. Others were somewhat longer than an oval, and these were very slow a-moving, and few in number. These animalcules had divers colours, some being whitish and transparent; others with green and very glittering little scales; others again were green in the middle, and before and behind white; others yet were ashen grey. And the motion of most of these animalcules in the water was so swift, and so various, upwards, downwards, and round about, that 'twas wonderful to see: and I judge that some of these little creatures were above a thousand times smaller than the smallest ones I have ever yet seen, upon the rind of cheese, in wheaten flour, mould, and the like.[1]

FIG. 1. Contemporary view (1667) of the source of the first collection of microorganisms. From reproduction in Collected Letters of Antoni van Leeuwenhoek, edited by a Committee of Dutch Scientists, 1939.

And so we have the discovery of the Protozoa and associated organisms, plant and animal—the first glimpse of a population for the most part beyond the ken of unaided vision. But, as we know, this was but the first of a long series of "population" studies by Leeuwenhoek, including the initial observations on vegetable infusions of

[1] From a letter written at Delft, September 7, 1674, and published, in part, in the *Philosophical Transactions of the Royal Society of London*, Vol. 9, No. 108, November, 1674. Quoted from the translation by Dobell in "Antony van Leeuwenhoek and his 'little animals,'" pp. 109–111, 1932. Also see the translation in "The collected letters of Antoni van Leeuwenhoek," edited by a Committee of Dutch Scientists, Vol. 1, pp. 162–165, 1939.

various sorts. His investigations on "pepper-water," the prototype of all later studies on "hay infusions," resulted in the discovery of Bacteria in 1676.

Having made sundry efforts, from time to time, to discover, if 'twere possible, the cause of the hotness or power whereby pepper affects the tongue (more especially because we find that even though pepper hath lain a whole year in vinegar, it yet retaineth its pungency); I did now place anew about ½ ounce of whole pepper in water, and set it in my closet, with no other design than to soften the pepper, that I could the better study it. This pepper having lain about three weeks in the water, and on two several occasions snow-water having been added thereto, because the water had evaporated away; by chance observing this water on the 24th April, 1676, I saw therein, with great wonder, incredibly many very little animalcules, of divers sorts. . . .

The fourth sort of little animals, which drifted among the three sorts aforesaid, were incredibly small; nay, so small, in my sight, that I judged that even if 100 of these very wee animals lay stretched out one against another, they could not reach to the length of a grain of coarse sand; and if this be true, then ten hundred thousand of these living creatures could scarce equal the bulk of a coarse sand-grain.[2]

A somewhat regular sequence of organisms in infusions of one kind or another attracted the attention of the early devotees of the microscope, as is shown, in particular, by the following paragraph from a letter written in September, 1702, by a keen observer, whose anonymity still holds, who was led by the writings of Leeuwenhoek to make such studies:

In my observations of the *Animalcula in Waters* I have seen many of the same species in the several infusions, and even in Waters that have been exposed (especially at this time of the year) any time without any particular mixture, such as you find in the hollow of a Cabbage-leaf, or on the *Dipsacus*, etc., and I am confident that many of these are the same Creatures under different dresses. For I have noted such a regular process in them, and such a constant order of their appearance, that I am of opinion most of them are the product of the Spawn of some invisible *Volatile Parents*. . . .[3]

Most assiduous among the other pioneers with the microscope at this period was Joblot of Paris, who collected his observations of many years in the first separate treatise on animalcules, published in 1718. Joblot was the first to give names to the organisms he observed, and in most cases discovered, in infusions of roses, pinks, mari-

[2] Quoted from translation by Dobell, *loc. cit.*, pp. 131–133.

[3] *Phil. Trans. R. S. London*, Vol. 23, No. 284, p. 1366, 1703.

golds, mushrooms, tree bark and so on, and also to make intensive studies on hay infusions to determine the origin of their teeming populations. He gave the initial demon-

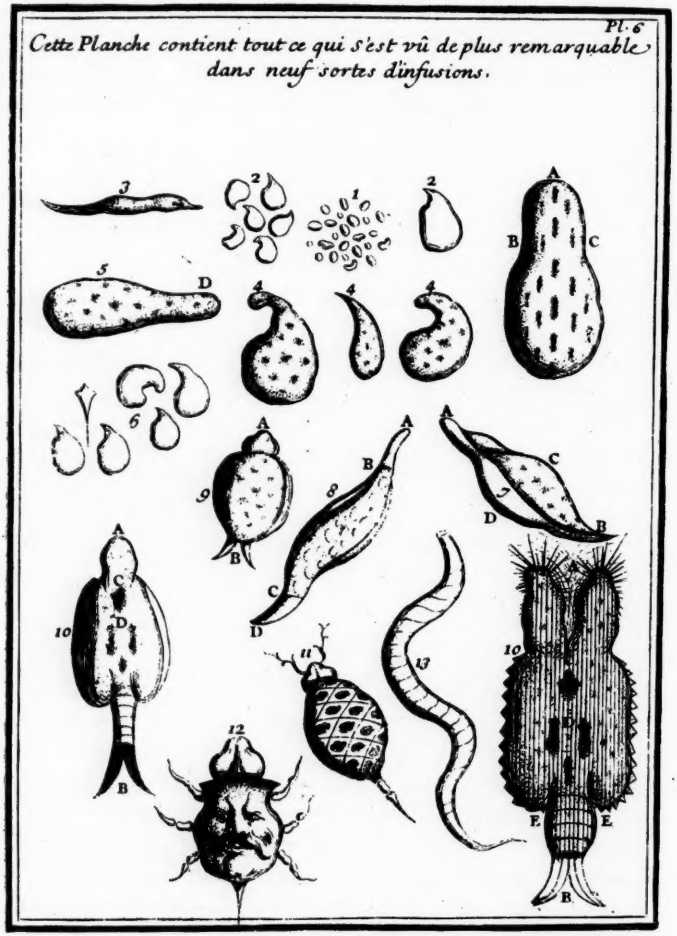

FIG. 2. One of the many plates in Joblot's pioneer treatise, 1718.

stration not only that organisms do not appear in infusions that have been boiled and sealed from atmospheric contamination, but also that such boiled infusions, though

devoid of life, again readily support life when admitted from the air. And it seems that this was the first use of heat as a method of eliminating life in such studies—a method exploited by Needham, Spallanzani and others nearly a half century later.[4]

Beyond the discovery of many new species of Protozoa and their classification as the most primitive animals by Hill, Muller, Ehrenberg and others, there is little to record until Dujardin, just one hundred years ago, initiated what may be regarded as the "modern" period in the study of protozoan populations with the quaint, but only too true, statement that "Rien de plus simple que de préparer des infusions et d'y voir se produire les Infusoires; mais rien de plus difficile que d'obtenir des résultats semblables de deux infusions préparées en apparence dans les mêmes conditions. . . ."[5]

This is neither the time nor the place to follow the details of Dujardin's work or that of the many investigators who, building on the foundations laid by the pioneers from Leeuwenhoek to Dujardin, have amassed the large amount of data now available in regard to populations of Protozoa under various conditions, natural and otherwise. We turn at once to a cross-section of present-day knowledge in the field as seen by a group of distinguished specialists. Professor G. E. Hutchinson, of Yale, discusses certain ecological aspects of succession in natural populations; Professor R. P. Hall, of New York University, and Professor W. H. Johnson, of Stanford, present, respectively, analyses of populations of green flagellates and of ciliates; Professor W. H. Taliaferro, of the University of Chicago, considers populations of blood-dwelling species; and, finally, Professor W. C. Allee, also of Chicago, integrates protozoan population problems with those of general biology.

[4] *Cf.* L. L. Woodruff, "Louis Joblot and the Protozoa," *Scientific Monthly*, Vol. 44, 1937.

[5] "Histoire naturelle des Zoophytes. Infusoires," p. 170, 1841. *Cf.* L. L. Woodruff, "Observations on the origin and sequence of the protozoan fauna of hay infusions," *Jour. Exper. Zool.*, Vol. 12, 1912, pp. 213–215.

ECOLOGICAL ASPECTS OF SUCCESSION IN NATURAL POPULATIONS

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THE striking changes that occur in both experimental mixed cultures and in natural populations have, as Dr. Woodruff has reminded us, been observed for a long time. In attempting a modern explanation of such changes it is first desirable to call attention to certain controlled laboratory experiments, that will provide a key to the more complex situation in nature. These experiments are due to Gause and are doubtless generally familiar; they are explicable by a relatively complete mathematical theory, provided by Lotka (1925), by Volterra (1926), and by Gause himself (1934, 1935). The particular experiments which are fundamental from the standpoint of the present contribution show that:

(1) If two species live in an identical niche, competing for the same food supply, maintained at a constant level, one species will entirely displace the other. This has been demonstrated with *Paramecium aurelia* and *Glaucoma scintillans;* the latter, having the higher coefficient of multiplication, alone remains.

(2) Dominance in competition is dependent on the environment conditions. This is strikingly shown in experiments with mixed cultures of *P. aurelia* and *P. caudatum,* in cultures that have, or have not, received biologically conditioned medium. If metabolic products of *Paramecium, Bacillus pyocyaneus,* etc., are not added, *caudatum* starts growing faster; if such products have been added, the reverse is true. In general, if the ecological conditions are such that utilization of food is the controlling factor, *caudatum* is dominant, if resistance to metabolic products, either hetero- or homotypical, is the controlling factor, *aurelia* is dominant (Gause, Nastukova and Alpatov, 1934).

The very elaborate development of Gause's work in which more than one niche is provided (*e.g.*, two foods of different sizes) need not concern us at present. The facts of fundamental importance are, firstly, the enormous difference that competition makes to organisms that singly exhibit superficially similar growth curves, and, secondly, that this dominance of one species over another is subject to environmental control. Comparable phenomena are known in other groups of animals, as for instance, the triclads (Beauchamp and Ullyott, 1932).

Successional changes have been studied primarily in systems of two kinds.

(1) Hay infusions, in which the free energy of the system is at a maximum at the moment of initiation of the experiment, and declines continually, though with presumably a decreasing rate.

(2) The open water of lakes and the ocean. The bioceonoses contained in such biotopes constitute machines causing a detour in the degradation of radiant energy from the sun. In the absence of seasonal changes the free energy might be expected to be constant, representing a steady state of production and decomposition of organic material, and a function of the supply of nutrient elements. In practice the free energy oscillates about a mean value. Such oscillations are largely seasonal, the mean value doubtless depending largely on the trophic standard, though the relations are very complex.

Any system in which external (seasonal) or internal changes modify properties which are involved in the struggle for existence will cause a successional series. In the hay infusion a number of workers (Fine, 1912, Bodine, 1921, Pruthi, 1927, Eddy, 1928, Darby, 1929) have shown marked changes in alkalinity, pH, CO_2 content, and by inference, O_2 content. These changes are obviously primarily determined by bacterial metabolism of the hay, the running down of the system, but differ in infusions with and without Protozoa. Both changes in oxygen content and in pH are probably involved in suc-

cession. The hay infusion, as commonly prepared, is emphatically not a single niche system (*cf.* Woodruff, 1912, Eddy, 1928) and the competition phenomena are liable to be complex. Metabolic products undoubtedly play an important part, either directly, or indirectly, in altering pH, but apart from Woodruff's pioneer work, their significance for infusion succession is largely inferential.

Somewhat simpler conditions are provided by the open waters of lakes and the ocean. Here we avoid the complexities of a solid substratum. A marked light gradient, a temperature gradient, and some chemical gradients occur, but by confining our attention to the epilimnion or epithalassa which is very thick compared with the length of a protozoan or, in most cases, the vertical component of its mean path, we can avoid consideration of spatial changes in chemical or thermal properties, so we have, at any moment, what is as close as possible to a single niche. The properties of this niche, however, vary seasonally.

In considering the populations of lakes and of the ocean, the most interesting forms are certainly autotrophic, particularly the Dinoflagellates and certain Chrysomonads, such as *Dinobryon*. Emphasis may be placed on these groups as they are not to be considered in great detail by later contributors to this symposium. Since such organisms must be supposed to enter into competition with other autotrophic members of the plankton, particularly diatoms and blue-green algae, it is impossible to discuss their periodicity without reference to that of the non-flagellate groups. The primary requirements of all these forms relate to *light, temperature,* the supply of *nutrient elements,* of which N, P, Fe, Mn and in the case of diatoms, Si, are the most important, and sometimes the presence of *organic accessory substances,* which may be regarded as equivalent to, and in certain cases are identical with, the vitamins of importance in vertebrate nutrition.

The annual cycles of light and temperature, being both dependent on the annual variations in the position of the sun, are in general similar, but since heat can be stored as molecular motion, whereas light can not, the rate of change of temperature and of light intensity is somewhat different in the spring, and minor fluctuations in the weather are more clearly reflected in the variation of light than of temperature. This makes it possible to separate by statistical methods their effects in nature. It would appear that wherever this has been done in considering the general spring increase of autotrophic plankton, the effect of light is at least as, if not more, significant than that of temperature. Almost fifty years ago Calkins (1893) noted the increase of diatoms during the "period of lengthening days, in spite of the almost freezing temperature of the water." An example of a more recent statistical study is provided by Riley's (1941) investigation of Long Island Sound. Richards (1929), moreover, believes that there is a statistically recognizable light factor influencing the division rate of pure cultures of ciliates (*P. aurelia, Blepharisma undulans* and *Histrio complanatus*). There is further evidence that even within a single genus the vertical distribution of populations is controlled by the illumination. This is most strikingly demonstrated by the *Ceratia* of the open ocean (Steemann Neilsen, 1934), where the depth distribution depends on the total amount of plankton shading the upper water. Many species of the genus are therefore shade species in just the same sense as are the plants composing the ground cover in a tract of woodland. In view of these various observations it seems not improbable that seasonal variation in light intensity may directly or indirectly play a rôle in determining succession of single species. Unequivocal separation of the effect of light and temperature in regulating seasonal replacement of one species by another does not yet seem to have been demonstrated, in spite of its inherent probability.

Turning to the variations in chemistry in the surface

layers of lakes and the ocean, we find complex but well-marked seasonal changes. These are largely determined by the organisms themselves. In general the increasing light and temperature in the spring lead to an outburst of photosynthetic plankton, which, in the epilimnion causes an increase in O_2 concentration and pH, a fall in the concentration of nitrate and phosphate. These changes are most marked in small lakes, but here subsequent events are irregular, though there is usually a marked fall in total plankton at midsummer followed by a rise of Cyanophyceae about the time of maximum water temperature. There is evidence that this latter event accompanies in some cases intense fixation of atmospheric nitrogen. In large lakes and in the sea there is generally an autumnal rather than an aestival secondary bloom, correlated with the breakdown of thermal stratification. In surface waters changes in oxygen concentration are probably of little importance; changes in oxygen tension in the water of lakes are of greater protozoological interest in the hypolimnion, where the reduction of O_2 during the summer, permits the development of a remarkable ciliate fauna.

Owing to the supposed ease with which the concentration of hydrogen ions can be determined by dropping solutions of dyes into water samples, pH became a fashionable symbol. It is, however, exceedingly doubtful if more than a single case has been brought forward demonstrating unequivocally that the natural variation in numbers of any species of animal is due to variation in pH, though it is not improbable that a number of ciliates in relatively saprobiotic habitats are affected by this factor. The work of Saunders (1924) and of Darby (1929) does indicate that *Spirostomum ambiguum* is really limited in nature by rises in pH over 7.8 and that comparable phenomena are probably exhibited by other species. Saunders's experiments are particularly important in that the pH was controlled both by Sørensen buffers and by calcium bicarbonate and CO_2, so that the effects are shown

to be independent of all ions save $H^{\cdot}$ and OH'. Numerous further examples of apparently acid or alkali water animals are known, but it has yet to be demonstrated that these are not due to variations in Ca or CO_2 content rather than $H^{\cdot}$. The apparent ecological insignificance of hydrogen ion concentration, in the numerous euryionic species that are known, contrasts so strongly with the immense and universal importance of this variable within the organism, that one is tempted to conclude that some independence of external variations in pH was one of the first prerequisites in the evolution of higher organisms. However this may be, the seasonal changes in pH, CO_2 or Ca in the open waters of lakes and the ocean are in general too small to be of importance in regulation of natural succession.

Combined nitrogen and phosphate are of paramount importance to all autotrophic forms. It is first important to remember the very great dilutions at which these substances occur. Concentrations of 100 γ per litre N or 20 γ P per litre are to be regarded as high; frequently even at times of increase in the autotrophic plankton, not more than 20–40 γ $N.NO_3$ and 0.5–2 γ P can be detected.

Secondly, it is clear that the space provided by the illuminated waters of lakes and the ocean is rarely filled to capacity. Only in the production of intense water blooms is any spatial crowding probable and in almost every case addition of appropriate substances to water under natural conditions of illumination will increase the crop. The autotrophic forms therefore live under intense conditions of competition for nutrient substances. It should also be pointed out that a relatively few divisions are needed to raise a species to a dominant position. One cell per cc. is usually below the limit of accurate counting, even though it may correspond to a population of 10^{10} cells in the top meter layer of a small lake. Yet after ten divisions a species at a concentration of one cell per cc. will be present, if all descendants survive, in a concentration of over 1000 per cc. and may well

have become a dominant, if the other species present are dividing more slowly or are surviving poorly.

Little information exists as to the optimal nutrient concentrations required by autotrophic plankters. The most recent and probably the most accurate work is that of Ketchum (1939), who found that reduction of nitrate nitrogen to as low a concentration as 50 γ per litre did not retard the growth of *Nitzschia closterium* populations. Reduction of phosphate phosphorus, however, below 17 γ per litre, did cause a marked fall in division rate of this marine species. Such a quantity is much above the maximal amount of free phosphate phosphorus (often less than 10 γ per litre) present in many lake waters, so that it is highly probable that differences in the minimal concentration required for maximal division would be found if different species were compared. If this is so, it is obvious that a mechanism exists for the production of succession during the period of nutrient exhaustion after a maximum of any species, for as the concentration falls, the species responsible for the depletion, which dominates at an initial high nutrient level, will suffer from competition with forms dominant at low nutrient levels. Pearsall's (1932) field data strongly suggest that the replacement of the diatom *Asterionella* by *Tabellaria* in the English lakes represents such a shift in population due to change in nutrient level. In Linsley Pond, North Branford, Connecticut, where *Tabellaria* is not of importance in the plankton, the appearance of *Asterionella formosa* is not regulated in the way indicated by Pearsall. In considering combined nitrogen as a primary nutrient it is important to bear in mind that it may occur in many inorganic forms; ammonia and nitrate are the most important, though nitrite and perhaps hyponitrite may also occur (Cooper, 1937). In some lakes (*e.g.,* Lake Mendota) the balance between bacterial production of ammonia, nitrification, and denitrification produces a stationary concentration of ammonia greater than that of nitrate; in other lakes

(English lake district and northeast Wisconsin) the reverse is true. Other cases occur in which the ratio of nitrogen as ammonia to nitrogen as nitrate shifts with the seasons (Yoshimura, 1932). Zobell (1935) and Harvey (1940) have shown that marine diatoms use ammonia in preference to nitrate when both forms of nitrogen are supplied. It is therefore probable that in waters with a low $N.NH_4 : N.NO_3$ ratio, variations in ability to use nitrate effectively may be of importance in regulating competition.

It is worthwhile noting that in any case (*i.e.,* the ocean or a large lake) in which a certain concentration of nutrients is established at the spring over-turn or full circulation, which concentration can not be maintained by additions during the summer period of utilization, the maximum population would inevitably be one developed largely of high nutrient forms. If, however, a slow production of nutrients is possible from the mud of shallower parts of the lake, as is certainly the case in small lakes (Hutchinson, 1941), a steady state can theoretically be established, leading to a peak in the production of species adapted to any nutrient level.

In relation to the thesis that competition for nutrients is of paramount importance in regulating succession, we may examine the seasonal incidence of the colonial Chrysomonad *Dinobryon.* In a large number of lakes, several species of this genus show a well-marked maximum after the spring phytoplankton bloom. Pearsall suggested that this was determined by a fall in the SiO_2 content of the water below 0.5 mgrs. per litre and by a rise in the N/P ratio to over 40 or so, the exact value being dependent on the silicate and calcium content. In Linsley Pond, where the Ca is always high and relatively constant, we have found *Dinobryon* (mainly *D. divergens*) to develop a maximum with a SiO_2 content of over 7 mgrs. per litre, and although the maximum is usually preceded or accompanied by a rise in the N/P ratio, this may be but little over 40 even with such a high SiO_2 concentra-

tion. Moreover, a study of the literature of the occurrence of this organism indicates that, while its incidence at periods of low SiO_2 is not confined to Pearsall's localities, remarkable exceptions to all of Pearsall's determining factors are known. It is noteworthy that *Dinobryon* maxima may intervene after a phase composed of either diatoms (*Asterionella* and *Tabellaria*) or of Cyanophyceae, groups which are probably very different biochemically; the production of some specific substance favoring *Dinobryon* by the antecedent species appears therefore unlikely. *A priori* it may be expected that in any water in which the N/P ratio is above the mean biological ratio of about 7, increasing utilization increases the ratio; this is confirmed by Ketchum's experiments, though in the inverse situation, an abnormally low N/P ratio is brought nearer to normal by absorption of nutrients. There are, moreover, usually great quantities of diatoms in the spring bloom. It is obvious that an increasing N/P ratio and a declining silicate content may both be symptoms of depletion. Where such symptoms are often, but not invariably, correlated with a change in the population, it seems natural to suppose that competitive relationships are involved. Owing to the complexity of such relationships, particularly when the species of competitors and the values of other factors regulating competition are not constant, we should hardly expect any hard and fast limiting value in the nutrient level to determine the incidence of *Dinobryon*.

Apart from combined nitrogen and phosphorus, iron and manganese are probably the most important inorganic substances to be considered. Iron raises special problems. In simple ionic form it is practically insoluble at the pH normally encountered in both fresh and salt waters. Suspended and colloidal ferric hydroxide are probably always available, and at least in fresh waters soluble or colloidal organic compounds also. The ferric hydroxide of fresh waters may well be normally present partly in the form of bacterial sheaths. Harvey has

shown that at least some diatoms can utilize suspended and colloidal iron; practically no information exists as to a possible seasonal influence. In the case of Mn, however, Harvey finds that not infrequently summer sea water which will not support the winter diatom *Ditylum brightwellei,* even after enrichment with N, P, and Fe, can produce a good growth if Mn be added. Mn depletion, therefore, is probably involved in the seasonal cycle of the sea.

More and more attention is now being given to those accessory organic substances that would be called vitamins in vertebrate biochemistry. Though primarily known by their effects in laboratory experiments, it seems desirable to consider briefly their possible rôle in natural environments. Harvey, again working with *Ditylum,* finds two groups of substances which he calls A (adsorbable on charcoal) and N (not adsorbable on charcoal) which are necessary for growth of this diatom. These may be absent from enriched summer water. Both can be derived from soils and from decaying sea weed. Substance "A" can be replaced by cystine, and by smaller amounts of thiamin (= vitamin B_1). A crude biotin (= vitamin H = coenzyme R) preparation was also effective. The natural "A" can not be thiamin, and *a fortiore* not cystine, as the amount (.25 mgr. per litre) required is disproportionate to the organic content (5 mgrs. per litre) of sea water. It is, moreover, apparently not an amino acid. Substance "N" can be replaced by α-alanine, lactic acid, gluconic acid, and dextrose. Harvey suspects its function is to form a soluble iron or manganese complex.

That these substances are apparently sometimes present, sometimes absent, in the sea, indicates that they may have an effect in the production of seasonal succession. A matter of particular ecological significance is raised by them because they may explain the otherwise puzzling fact that the depletion of combined nitrogen and of phosphate often goes much farther in small eutrophic lakes

than in oligotrophic lakes or in the sea. It is very usual to find, therefore, large crops of plankton continuing to develop at a nutrient level apparently less satisfactory than that provided by water which never produces much planktonic growth. Moreover, great differences in vitamin requirement are already known in protists, fungi, and bacteria, and not improbably many puzzling facts in distribution may find their explanation here. Steemann Nielsen suggests on the contrary that organic matter in neritic waters excludes many species of oceanic *Ceratia*. It is, however, possible that competition with otherwise more successful forms which require a higher amount of accessory substances is involved.

Finally a word must be said about relationships between prey and predators, as this concerns holozoic forms. As is well known, Lotka and Volterra have shown that cyclical variation in the numbers of two forms, one feeding on the other, can theoretically be set up and perpetuated. In any case, there are definite particular numbers of the two species which can coexist constantly at a singular point; however, if either number is altered no return to the singular point is possible, but a cycle is generated, so that the number of one species plotted against the other goes round and round the singular point. Gause, who studied this matter experimentally with *Didinium* and *Paramecium*, found that in a uniform microcosm no agreement with theory occurred. In general this is due to the fact that if predation is very efficient, the singular point lies so near the origin that chance statistical variations will soon bring one or other population to zero. If the predator is kept artificially rarified in proportion to its abundance, *i.e.*, if the predation is reduced, a well-developed cycle can be introduced into the system, as has been shown by Gause for *P. caudatum* feeding on *Schizosaccharomyces*. In most cases in nature it is probable that rhythmic fluctuations in numbers are not due to simple prey-predator relationships, though other types of internal rhythm may develop. Gause,

however, suggests that one remarkable example involving the protozoa is dependent on the prey-predator relationship, namely the striking variation in numbers of bacteria and of protozoa in soils, recorded by Cutler, Crump, and Sandon (1923).

In conclusion, it is apparent that a variety of environmental factors may control succession in protistan populations. In laboratory experiments clear-cut results can often be obtained; in nature a bewildering number of possibilities present themselves, and the further analysis is carried the more difficult is it to isolate controlling physico-chemical variables. This difficulty should be admitted as one of the relevant facts to be considered; it is indeed one of the most important of the data, for it strongly suggests that in many cases modification of the dominance exhibited among numerous competing species is the major rôle of the environmental factors, rather than the direct transgression of limits of tolerance, so easily studied in laboratory experiments with pure cultures.

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POPULATIONS OF PLANT-LIKE FLAGELLATES

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FLAGELLATES in laboratory populations are exposed to conditions somewhat different from those of natural environments. Temperature and other factors are usually controlled within favorable limits and food is usually abundant, so that growth-rate and density of population are often higher than under natural conditions. On the other hand, laboratory populations encounter certain limitations. The food supply of a pure culture, except when augmented by photosynthesis, may be limited to that originally present in the medium, and its depletion eventually checks growth. A pure culture is subject also to the accumulation of waste products which, under natural conditions, may be removed by dilution and by other organisms. In short, the laboratory culture, sometimes from the very beginning, may be undergoing changes which become increasingly detrimental to growth as the population increases in density. That such unfavorable changes in the medium are reflected in the division-rate of flagellates is indicated by Jahn's (1929, 1930) observations on *Euglena sp.* The division-rate of this flagellate in an inorganic medium was relatively high for the first 24 hours, but decreased steadily thereafter, tending to follow a sigmoid curve with a negative slope. When biological conditioning of the medium exerts a favorable action, the onset of detrimental changes may be delayed slightly, but the effects are none the less real when they do occur. The natural environment, in contrast to the laboratory culture, often approaches an equilibrium in which a given population may fluctuate about a characteristic level under the influence of moderate environmental changes.

The laboratory population sooner or later reaches

maximal density, becomes old, and then enters a phase of "accelerated death" (Buchanan). A growth-curve of this type has been reported for *Polytoma obtusum* by Provasoli (1938), who described phases of lag, logarithmic growth and maximal density, followed by a rapid decline in density of population. Likewise, populations of *Euglena sp.* (Jahn, 1929) and *Polytoma uvella* (Rottier, 1936) have been traced into the phase of maximal density.

Little is known concerning the early phases in growth of flagellate populations. In *Euglena sp.*, however, Jahn (1929, 1930) demonstrated that the division-rate decreased steadily after the first day of incubation. Unpublished observations of the writer indicate that the same thing may be true for two of the ciliates in certain media. In other words, the division-rate, after the first 24 hours or so, may decrease steadily throughout growth of a population. Such a division-rate suggests that the culture medium, in these cases, is satisfactory for rapid division of a small number of organisms, but becomes increasingly unsatisfactory as the population grows in density. The factors thus influencing division-rate have not been determined, although some evidence indicates that changes in carbon-dioxide content (Jahn, 1936), in oxygen-tension (Jahn, 1936; Rottier, 1936) and in redox-potential (Jahn, 1935) of the medium are important. Other possibilities obviously include the exhaustion of specific minerals—*e.g.*, calcium (Dusi, 1933; Pringsheim, 1937), iron (Lwoff, 1930; Schoenborn, 1940; Hutchens, 1940), manganese (Hall, 1937)—and vitamins usually present only in traces. When a lag phase occurs, it may represent a period in which favorable changes in the medium are being produced by the inoculated organisms —an effect often termed biological conditioning. Or, the lag phase may represent a period during which the inoculated organisms are becoming adjusted to a new medium or are recovering from the effects of unfavorable conditions encountered in the stock culture. Or a combination

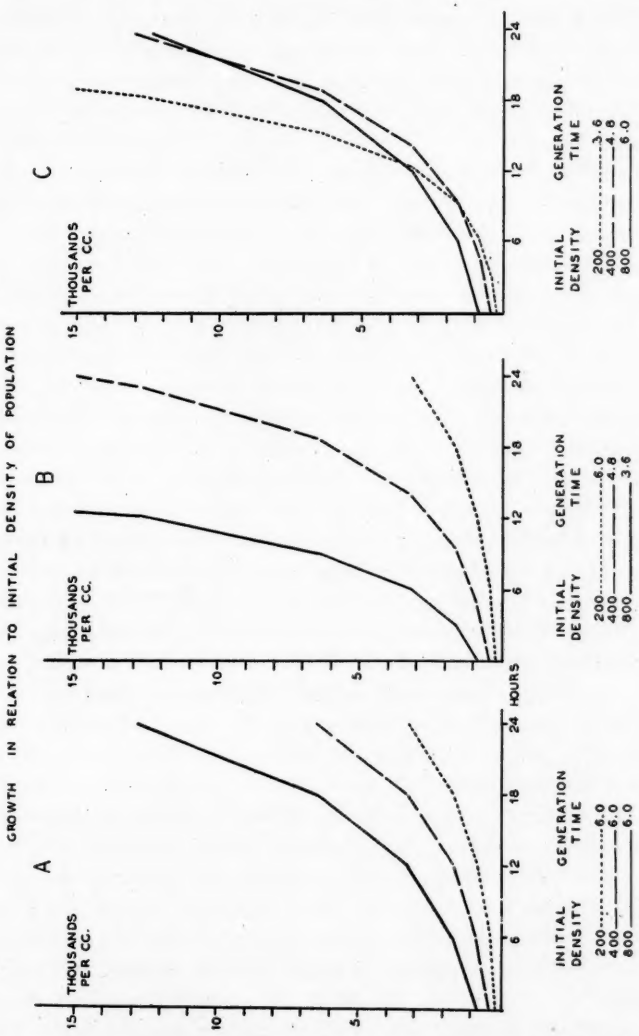

FIG. 1. Three possible relationships between growth-rate and initial density. Generation times (in hours) assumed arbitrarily for populations with initial densities of 200, 400 and 800 per cc.

of factors may contribute to the appearance of a lag phase. Such a culture shows an increasing division-rate for a time, but this phase is followed sooner or later by a steady decrease in division-rate, as in the cultures described above.

For any one species, both the density attainable and the time required for development of maximal density vary with the medium. In *Lobomonas piriformis* (Osterud, MSS), for example, maximal density in a casein-peptone medium may be reached in less than 4 weeks, while a population in gelatin medium may still be growing at the end of 18 weeks. In a particular medium, the time required for a population to reach maximal density varies with environmental conditions, and such environmental influences may be striking in strains carried through serial transfers. On the other hand, the growth-rate of a strain may vary, even under apparently constant conditions, and there is evidence that some of these variations may be correlated with differences in initial density of population.

Three possibilities are apparent for relationships between growth-rate and initial density of population.

(1) It may be assumed that division-rate remains the same no matter what the initial density happens to be (Fig. 1, A). Populations during the phase of rapid growth would differ merely according to the ratio of initial densities. The existence of such a neutral situation has not been demonstrated in plant-like flagellates.

(2) Or, the division-rate may be increased as the initial density of population is increased (Fig. 1, B). In this case, populations with different initial densities would diverge rapidly throughout the phase of rapid growth. This is the so-called *allelocatalytic effect,* first described by Robertson (1921) for ciliates. Under unfavorable conditions, it seems likely that cultures started with large inocula would have a better chance of survival than those receiving small inocula. Growth-rates might be influenced in the same manner, particularly in un-

favorable media which are readily modified toward an optimum by the organisms themselves. Allelocatalytic effects have been reported in *Chilomonas paramecium* (Mast and Pace, 1938) and, under certain conditions only, in *Euglena gracilis* (Sweet, 1939). Such allelocatalytic effects, in any case, would probably be limited to the early history of a population before the density reaches a point at which detrimental changes in the medium begin to depress division-rate. In other words, an allelocatalytic effect, when it actually exists, will probably be detected only in short-run observations on young populations.

(3) As a third possibility, the division-rate may be highest for cultures with the lowest initial density (Fig. 1, C). This is the *Woodruff effect,* described originally in *Paramecium* (Woodruff, 1911). This relationship between growth and initial density has been demonstrated in *Euglena sp.* (Jahn, 1929) and in *E. gracilis* (Sweet, 1939). The populations tend to converge during the phase of rapid growth in this case, in which a high initial density is a handicap to rapid growth. In cultures with an inadequate or barely adequate food supply, consumption of food might exert such an influence. Comparable results might be expected with progressive changes in oxygen-tension and similar conditions away from the optimum. In short, unfavorable changes in the medium, which are related to the density of population and are to be expected after appreciable growth of a population, may extend their action to the time of inoculation and thus produce the *Woodruff effect* in young cultures.

These illustrations are enough to indicate that the relationships between growth-rate and initial density of population may not be simple, since the factors modified by initial density are undoubtedly multiple, and to suggest that there is need for caution in the interpretation of experimental results.

Investigation of such problems has followed two general methods. In one procedure, the same number of organisms are introduced into cultures containing differ-

ent volumes of medium. The result is a series of cultures with different initial densities. It appears, however, that changes in total volume of medium may modify the growth-rate, even when initial density remains constant. In *Astasia,* data of Schoenborn (1940) show such effects of different volumes for initial densities of 50–370 flagellates per cc. The observations of Sweet (1939) demonstrate similar effects in *Euglena gracilis* (Fig. 2). For *Chilomonas paramecium* also, data of Mast and Pace (1938, Table 4) show that a difference in volume of medium, without a change in initial density, may be correlated with a change in division-rate. Basic factors underlying such changes in surface-volume relationships

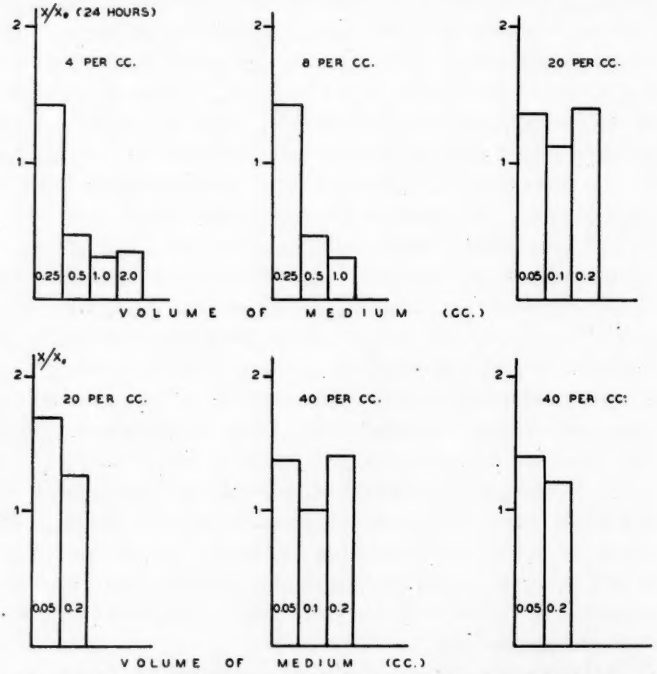

FIG. 2. Growth of *Euglena gracilis* for initial densities of 4–40 per cc in various volumes of medium; based on data presented by Sweet (1939) for different experiments with washed inocula. Growth is expressed as x/x_0 (ratio of final to initial density).

have not been investigated. However, it is obvious that the method of varying initial density by changing the volume of medium is not a reliable one for investigating the relation of growth to initial density.

In the second procedure, different numbers of organisms are introduced into identical volumes of medium. The validity of this method depends upon the technique of inoculation. If materials are transferred from stock culture to experimental cultures, there remains a possibility that initial density of population is not the only variable influencing growth-rate. If the medium is organic, materials from the stock culture might include, for example, products of extracellular digestion producing differential effects in cultures receiving different inocula. An inorganic stock medium might contain organic materials arising from death and decomposition of some of the organisms, or it might contain organic or inorganic metabolic products which would influence growth. This source of error can be eliminated by thorough washing of the organisms before inoculation. With this precaution observed, differences in division-rate may reasonably be attributed to differences in initial density.

For any one medium, it seems possible that there may be an optimal initial density for rapid growth of a species in young cultures. Below the optimum, an appreciable period of conditioning might be required before a slightly unsatisfactory medium could support rapid growth; or, with extremely small inocula, survival of the population might even be precarious. Above the optimum, the young population would become increasingly subject to the various unfavorable changes which are directly related to density of population. This question of an optimal initial density has been considered in *Chilomonas paramecium* (Mast and Pace, 1938) and in *Euglena gracilis* (Sweet, 1939).

In *Chilomonas paramecium,* Mast and Pace have reported that, with increasing initial densities up to an optimum, there is a corresponding increase in division-

rate (Fig. 3), or an allelocatalytic effect. Above the optimum, the relationship is reversed, and increasing the initial density progressively decreases the rate of growth (Woodruff effect). As indicated in Fig. 3, the optimum varied from 83 to 400 per cc in different volumes of

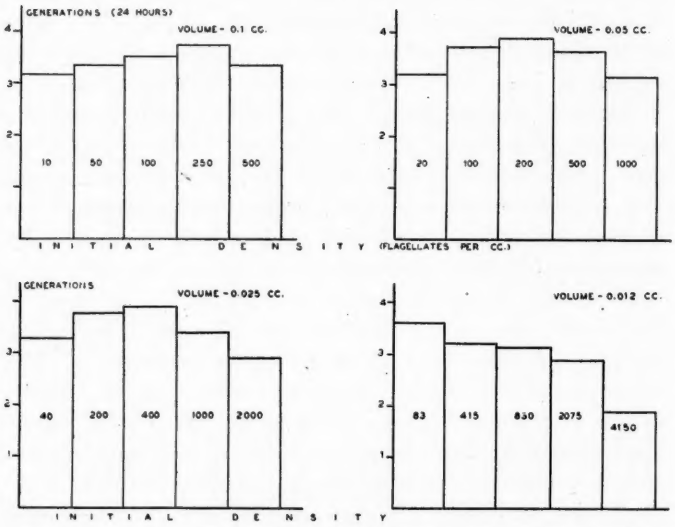

FIG. 3. Growth of *Chilomonas paramecium* for various initial densities in four volumes of medium; based on data presented by Mast and Pace (1938, Table 4).

medium. In each volume, the number of generations in 24 hours dropped as the initial density exceeded the optimum. On first inspection, the data suggest that the higher initial densities retarded growth to an appreciable extent.

However, calculation of final densities shows that cultures of *Chilomonas* with the highest initial densities actually developed the heaviest populations. With initial densities of approximately 2,000–4,000, the populations at 24 hours exceeded 15,000 per cc. With initial densities of only 200–400 per cc, the optimum in three cases, populations ranged from 2,744 to about 6,000 per

cc. These results are logical if they are interpreted in terms of populations.

It may be assumed that some hypothetical flagellate reaches a maximal density of 16,000 per cc in a certain medium. Assuming an average generation-time of 6.5

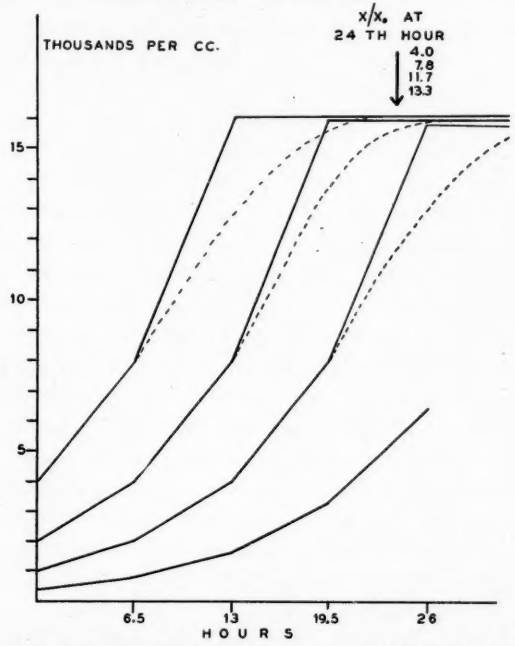

FIG. 4. Growth-curves for populations of a hypothetical plant-like flagellate; initial densities: 4,000; 2,000; 1,000; 400 per cc. Generation-time is assumed to be 6.5 hours in young populations; growth is expressed as x/x_0 (ratio of final to initial density) at the end of 24 hours.

hours, point-to-point curves may be plotted for initial densities of 4,000, of 2,000, of 1,000 and of 400 per cc (Fig. 4). At 24 hours, as indicated by the solid lines, the population is at maximal density in the first two cultures, and is approaching maximal density in the third. However, it is known that growth-rate decreases as such a population approaches maximal density, and it may be assumed that 8,000 per cc, as an arbitrary figure, repre-

sents the turning point for this flagellate. The corrected curves would tend to approach the dotted lines, and the corresponding growth-rates (x/x_o) at 24 hours would approximate 4.0, 7.8, 11.7 and 13.3.

Chilomonas, with an initial density of 4,150, developed a population of 15,604 per cc, while growth-rate was 3.76. Cultures with initial densities of about 2,000 developed almost the same population, although growth-rate was approximately doubled. And so on, for the lower initial densities down to the optimum. In other words, Mast and Pace may have shown that populations of *Chilomonas* with high initial densities reach or approach maximal density sooner than do populations with relatively low initial densities. This would not demonstrate that initial densities above a supposed optimum actually depressed division-rate, since comparison of division-rates would not be valid if based upon some populations which were actively growing and others in which growth had already ceased. Consequently, the data of Mast and Pace fail to establish either an optimal initial density or a Woodruff effect for cultures of *C. paramecium.*

In the case of *Euglena gracilis,* maintained in a rather unsatisfactory inorganic medium, Sweet (1939) suggested an optimal initial density lying within the limits of 4 and 50 flagellates per cc. This suggestion is in accord with the data presented. It is obvious, of course, that this optimal initial density applies only to the medium of Sweet, under the specified conditions of incubation, and that in a different medium or under different conditions of incubation *E. gracilis* might show a different optimum.

The Woodruff effect—involving a growth-rate more or less inversely proportional to the initial density of population—has been reported in *Euglena sp.* (Jahn, 1929), *E. gracilis* (Sweet, 1939) and *Chilomonas paramecium* (Mast and Pace, 1938).

In Jahn's investigations on *Euglena,* tube-cultures were inoculated with different numbers of washed flagel-

lates. In three experiments, involving initial densities ranging from 55 to more than 9,000 per cc, growth-rate was highest for cultures with the lowest initial density. In experiments with washed inocula, Sweet (1939, Figs. 1, 2) likewise observed that growth-rate of *E. gracilis* was highest for the lowest initial density in small volumes (0.05–0.2 cc) of medium. Accordingly, it may be concluded that the Woodruff effect occurs in populations of *Euglena,* at least under certain conditions, and that the effect may be evident after only 24 hours of incubation. In *Chilomonas,* however, the data of Mast and Pace fail to demonstrate a Woodruff effect, as pointed out above.

An allelocatalytic effect, apparent as a higher growth-rate for the higher initial densities, has been reported in *Euglena gracilis* (Sweet, 1939) and in *Chilomonas paramecium* (Mast and Pace, 1938).

Sweet observed an allelocatalytic effect, with washed inocula, in cultures containing 0.25 cc of medium. With initial densities of 16–32, growth-rate was significantly higher than with initial densities of 4–8 per cc (Sweet, 1939, Fig. 1). These results reversed those obtained in smaller volumes of medium. Likewise, after ultraviolet irradiation, the Woodruff effect was reversed, more or less completely, in 0.05 cc of medium, while in 0.2 cc of medium irradiation produced no marked effect for the higher initial densities but reduced growth-rate progressively for the lower initial densities (Sweet, 1939, Fig. 2). Interpretation of the latter results is complicated, as Sweet pointed out, by the injury to irradiated flagellates and also by the fact that irradiated cultures received more material from the stock cultures than did those started with washed inocula. However, it seems clear that the highest initial densities have a real survival value during irradiation. These results of Sweet are particularly interesting, since they demonstrate that the relationship between growth-rate and initial density may be modified by environmental conditions.

In "non-sterile" cultures, containing bacteria from hay

infusions, growth occurred with the higher initial densities but not with the lower. The results demonstrate a survival value of high initial densities in bacterized media, and suggest a possible allelocatalytic effect under such conditions. Accordingly, it appears that cultures of *E. gracilis,* under the conditions specified by Sweet, may exhibit a definite allelocatalytic effect.

In *Chilomonas paramecium,* Mast and Pace have concluded that, for initial densities below a supposed optimum, there is a consistent allelocatalytic effect. The evidence presented in their Table 2 is of questionable value. The technique employed—varying the volume of medium for a constant inoculum—is not an adequate test for allelocatalysis, and the differences in growth-rate which Mast and Pace have attributed to differences in initial density are comparable to the effects which they produced (1938, Table 4) by changes in the volume of medium alone.

In other experiments (Mast and Pace, Tables 1, 4), however, the inoculum was varied for a constant volume of medium. For initial densities below the "optimum," division-rate was highest for cultures with the highest initial density; lowest, with the lowest initial density (Fig. 3). Accordingly, it seems that division-rate was increased progressively as more and more flagellates were introduced into a small volume of medium. These results suggest an allelocatalytic effect. However, the significance of the results is uncertain. Unlike Sweet, Mast and Pace did not wash their flagellates thoroughly before inoculation of experimental cultures, and their methods would have insured transfer of conditioned medium from the stock culture—small amounts with one or a few flagellates; larger amounts with the introduction of many flagellates into experimental cultures. Hence, the initial density of population was not the only important variable in their experiments.

The relation of initial density to survival of *E. gracilis* under a variety of conditions has been investigated by

Sweet (1939). In general, her results demonstrate that the higher initial densities have a definite survival value for flagellates in bacterized medium, for washed flagellates in certain volumes (0.5–2.0 cc) of sterile medium, and for flagellates exposed to ultraviolet irradiation.

The question of an *autocatalyst of growth* has been considered by Jahn (1929, 1930), by Sweet (1939) and by Mast and Pace (1938). Jahn's results failed to demonstrate an allelocatalytic effect or to supply evidence for the existence of an autocatalyst of growth. Sweet, although observing an allelocatalytic effect under certain conditions, concluded that her results did not justify acceptance of Robertson's theory of an autocatalyst. Mast and Pace, on the other hand, have concluded that the observed differences in division-rate of *Chilomonas* are dependent upon "X-substance," which is produced by the flagellates and accumulated in culture fluid or stored in the protoplasm. "X-substance" is said to accelerate growth quantitatively, when present in favorable concentrations, and to depress division-rate when present in excess.

In order to explain the apparent decrease in growth-rate with initial densities above the supposed optimum, Mast and Pace tested the effects of "X-substance" in excess. As recorded in their Table 7, depression-slides containing fresh medium, "old" fluid from 6-day cultures and boiled "old" fluid were inoculated with single flagellates. No division occurred in "old" fluid until after 8 hours, and less than two generations in 22 hours. In boiled "old" fluid, however, the division-rate approached that in fresh medium. It was concluded that "old" fluid, in which a population of *Chilomonas* has already died, contains so much "X-substance" that division is retarded. Boiling for 10 seconds or longer progressively destroys "X" and permits normal growth.

In other experiments, Mast and Pace (Table 8) compared growth of *Chilomonas* in "young" fluid (containing 35 units of "X") with that in fresh medium and in

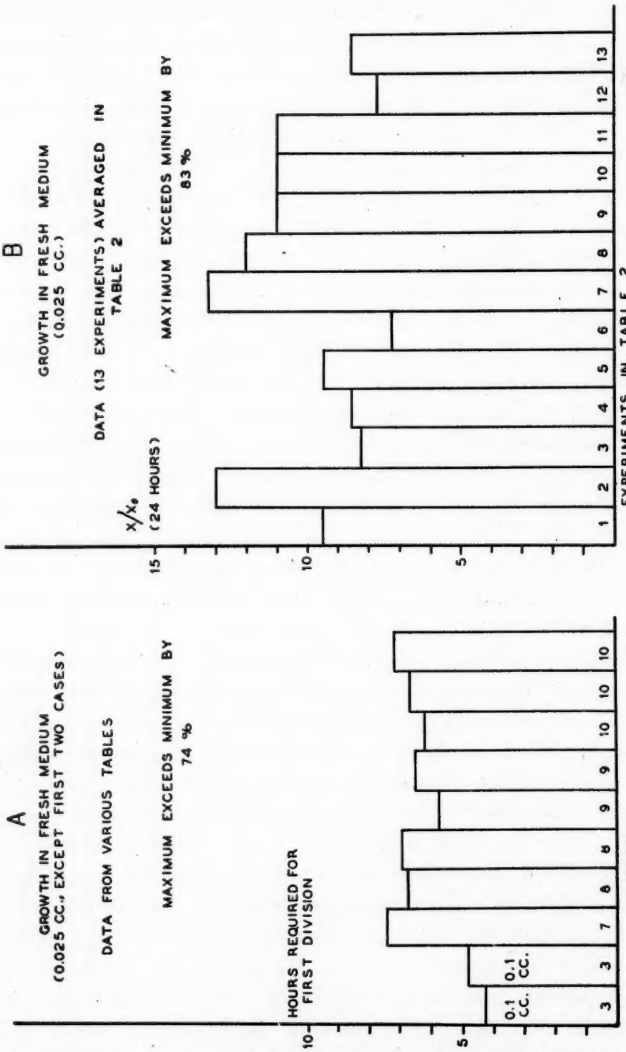

FIG. 5. A. Hours required by *C. paramecium* for completion of first division in fresh medium; based on data from Tables 3, 7–10 of Mast and Pace (1938). B. Growth (x/x_0) of *C. paramecium* in fresh medium; based on data from Table 2 of Mast and Pace.

boiled "young" fluid. Their results were interpreted as indicating that a low concentration of "X," as found in "young" fluid, accelerates division, while boiling the "young" fluid destroys "X" and reduces division-rate to that in fresh medium. Similarly, the effects of "medium" fluid, containing 150 units of "X," were determined (Mast and Pace, Table 9). It was concluded that "medium" fluid contains an excess of "X," which depresses division-rate. Boiling for 15 minutes reduces the concentration to an optimum; with longer boiling, all of the "X" is destroyed and the division-rate approaches that in fresh medium.

However, the results obtained with "young" and "medium" fluids are less consistent than the conclusions of Mast and Pace imply. Comparison of data for "young," "medium" and fresh fluids shows that the effects of the first two were purely relative. In two groups of cultures (Table 9, A; Table 10, B), "medium" fluid, said to depress division-rate, supported more rapid first divisions than "young" fluid (Table 8, A, B) which is said to accelerate division. In a third group (Table 9, B), division in "medium" fluid was about the same as in the slowest group of controls in fresh medium (Table 10, A).

The results for the first division in "young" and "medium" fluids also seem less significant when compared with data for cultures in fresh medium (Fig. 5, A). Two groups of cultures in 0.1 cc of medium are included here for comparison with others in 0.025 cc. The comparison should be conservative, however, since Mast and Pace have concluded that division is slower in the larger volume with an inoculum of one flagellate. In these data, the range of variability for completion of the first division is about three hours, and the maximum exceeds the minimum by 74 per cent. Since the effects ascribed to "young" and "medium" fluids amount to differences of only 4–19 per cent., as compared with their respective controls, it would seem that the data of Mast and Pace fail to distinguish clearly between the normal variability

of *Chilomonas* and the action of "X-substance" in low concentrations. However, it may be unfair to compare the tests on "young" and "medium" fluids with other groups of experiments, since the variation for several groups might be much greater than that within a single group. This possibility may be tested with the data (0.025 cc of medium) in Table 2 of Mast and Pace. These 13 experiments (Fig. 5, B) were considered comparable, and conclusions were based on an average for the 13 groups of cultures. Here, the average growth-rate ranged from 7.2 to 13.2, the maximum exceeding the minimum by 83 per cent. Hence, the variability of *Chilomonas* under comparable conditions, as recorded in Table 2, is of about the same order as that recorded in Tables 3, 7, 8, 9 and 10. Accordingly, it is impossible to conclude that Mast and Pace have demonstrated the existence of an "X-substance" in low concentrations in "young" and "medium" fluids.

In experiments of a second type (Fig. 6), Mast and Pace have presented evidence that "X-substance" is stored in the protoplasm of *Chilomonas*. The "strong" lines were inoculated from a 6-day stock culture; the "weak" lines, from a 2-day culture started with 1 flagellate; the "medium" lines, from a 2-day culture started with 500 flagellates. In experiment A, the lines were maintained in the same depression slides, one flagellate being discarded after each division; in experiment B, one flagellate was transferred to a fresh depression, and the other discarded after each division. The first division was slowest in the "strong" lines and fastest in the "weak" lines. It was concluded that the "strong" flagellates had accumulated so much "X" in their protoplasm that division was prevented until diffusion into the medium had reduced the protoplasmic concentration to a favorable one. With further diffusion, later divisions occurred more rapidly as the protoplasmic concentration of "X" approached an optimum. "Weak" flagellates, on the other hand, contained so little "X" that the first division was not delayed.

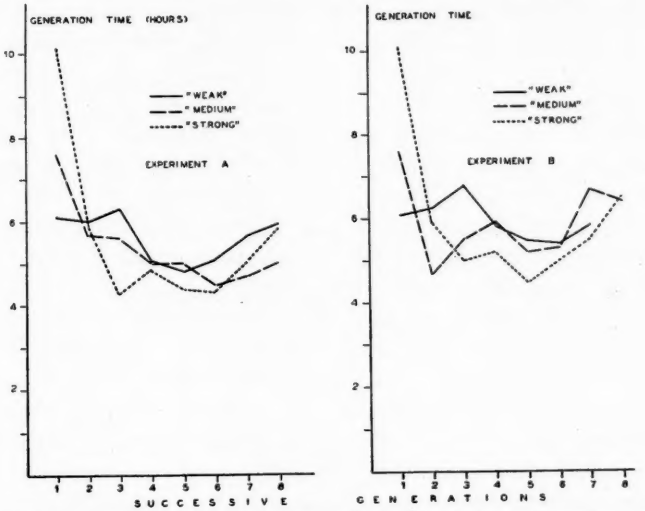

FIG. 6. Growth of *C. paramecium* in lines started from different types of stock cultures; based on data in Table 11 of Mast and Pace (1938).

The results may also be interpreted on the basis of differences in initial stationary phase. Since Mast and Pace have stated that mass cultures of *Chilomonas* die out in about 6 days, the "strong" lines obviously were started from old stocks and might be expected to show a pronounced initial stationary phase. The "weak" lines, from young stocks, might show no stationary phase at all. The "medium" lines, from a stock of intermediate physiological age, might show an intermediate stationary phase. After two or three generations, division-rate might be expected to approach a normal level in all three lines.

Obviously, it may be assumed that an initial stationary phase and a lag phase as well, in cultures started with old inocula, are caused by the accumulation of some substance or substances in the protoplasm of flagellates. On the other hand, such an assumption may not be necessary for explanation of these phenomena, and the mere occurrence of an initial stationary phase or of a lag phase does

not constitute evidence that "X-substance" is stored in the protoplasm of *Chilomonas* or that "X-substance" even exists. Accordingly, it is impossible to avoid the conclusion that Mast and Pace have failed to demonstrate that an "X-substance," supposedly produced by *Chilomonas,* is stored either in culture fluid or in the protoplasm of the flagellates.

In summarizing the evidence derived from the study of plant-like flagellates, it may be pointed out that relatively little is known about the behavior of populations. Practically nothing is known concerning growth during the first 24 hours of incubation, althought the paper of Mast and Pace (1938) contains a few data which, if analyzed, might throw some light on the growth of very young populations. In the only detailed analysis of growth in populations traced for several days, Jahn (1930) has shown that growth of *Euglena* tended to follow the autocatalytic curve, and this would automatically preclude the type of growth attributed by Robertson to an "autocatalyst." In *Euglena gracilis,* Sweet (1939) has shown that relationships between growth-rate and initial density may vary with environmental conditions. Under certain conditions, relatively high initial densities may be correlated with higher growth-rates and may also show a real survival value. Under other conditions, the lower initial densities are correlated with the higher growth-rates. The results of Mast and Pace suggest that conditioned medium, transferred from stock culture to experimental cultures, may favorably influence growth-rate, but their data fail to demonstrate the existence of an "X-substance" with quantitative effects on growth. At present, the basic factors underlying the relations between growth and initial density remain to be determined.

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POPULATIONS OF CILIATES

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A FEW years ago Jahn (1934), in a discussion of problems of population growth in the protozoa, stated that a mathematical analysis of most of the data concerning growth of protozoan populations would not appreciably increase our understanding of the matter because, in most of the investigations in this field, the various factors which might affect the results were not adequately controlled.

The situation is much the same to-day. We are still in the process of acquiring information about the factors which operate in such populations and of obtaining control over these factors. But there has been progress. A survey of the investigations of ciliate populations made during the past thirty years reveals progress in the techniques employed. In the beginning, such studies were made using unknown mixed bacteria as the source of food; then known bacteria were used; then known bacteria in definite amounts suspended in a non-nutritive reproducible salt solution were used; and, finally pure culture, in which no other form of life was present other than the one species being studied, was attained. Most of the pure culture studies up to the present time have been made on a few species, but recent developments, which will be referred to later, point to an extension of the pure culture technique in the future.

In such population studies what are some of the questions for which answers are being sought? In general the fundamental problems for analysis in studies of ciliates are the same as those found in similar studies of other kinds of organisms. The question of what factors affect the rate of population growth and the question of what factors limit population increase have been the primary concern of most workers on ciliate populations. The question of what goes on in a stabilized population,

where the density remains relatively constant over long periods of time, with reference to the rate of reproduction and the death rate is an interesting one. So little has been done on this problem in the ciliate studies that it can not be discussed here. However, this is a definite problem for future work.

In obtaining answers to these questions an analysis of numerous factors is necessary. Important in such analyses are the effects of metabolic products, the effects of numbers of the ciliates themselves, and the effects of the food—the nature and the amount of both the basic food materials and the food accessories. There are other important factors which must be analyzed and controlled, such as the hydrogen-ion concentration of the medium, the oxygen tension, the carbon dioxide tension, temperature and perhaps others. However, in the short time allowed for this discussion it will not be possible to attempt an analysis of all the factors which may operate. This discussion will center about the problems found in studies on the effects of waste products on ciliate populations, on the effects of numbers of organisms on such populations, and on the effects of the food supply. These factors are interrelated and it is not possible to discuss each factor entirely apart from the others. An attempt is made to consider the effects of each factor separately, in so far as that is possible.

Since the pioneering work of Woodruff (1911 and 1913) the effect of metabolic waste products has been a much investigated subject. Woodruff found that the waste products of *Paramecium, Stylonychia* and *Pleurotricha* depressed the division-rate of the species which produced them, but that they had either no effect, or an accelerative effect, on other species. Woodruff obtained the depressing effect on division-rate when from 1 to 4 organisms had lived for 24 hours in about 0.5 cc of medium. Using volumes of media, periods of time and numbers of animals comparable to those employed by Woodruff, Myers (1927), Calkins (1926), Greenleaf (1926), Petersen

(1929), Di Tomo (1932) and Beers (1933) obtained depressing effects of excretory products on the division-rates of several different ciliates.

The conclusion of Woodruff that one species of protozoan may condition the medium so that it is a more favorable place for another species to live in has been verified by many workers. In fact, the succession that occurs in a protozoan culture of long duration is almost a classic study in biology and protozoology classes. But the conclusion of Woodruff and others that waste products of a given species have a depressing effect in a relatively short period of time may have to be somewhat revised on the basis of recent work.

Taylor and Strickland (1938) were able to grow *Colpoda duodenaria* in 500-cc cultures of balanced salt solution with a single kind of bacterium as the source of food with uniform growth rates. In the course of this work they allowed the population to grow, after the introduction of a given quantity of food, and then subside with most of the organisms encysting. The subsequent introduction of a similar quantity of food resulted in a comparable growth of the population. This practice was repeated a number of times during a 4-months period with the same results. They concluded that excretion products accumulating in the medium for 4 months, do not affect the growth curves.

Kidder and Stuart (1939) in studies on *Colpoda* in which they used as the source of food the bacterium, *Aerobacter,* suspended in distilled water, obtained results which show that excretory substances appear to play a small rôle in growth limitation of *Colpoda.* They report that they found no apparent effect of metabolic waste products of either the protozoa or food organism in cultures where the ciliates reached a concentration of 20,000 per 0.005 ml.

Johnson and Hardin (1938) in a series of experiments on *Paramecium multimicronucleatum* tested the effects of old culture medium on division-rate of this ciliate over

different periods of time varying from 2 weeks to 1 month. In these experiments the same medium was used over and over. The paramecia were removed each day and fresh food and a new seeding of paramecia were introduced. In all of these tests there was no significant difference between the rate of reproduction in the conditioned cultures and in those started daily in fresh medium. During this investigation the effects of bacteria-conditioned medium on the division-rate of *Paramecium* was studied. The conditioned medium in this case was made by adding daily a loopful of bacteria to a culture dish containing 5 cc of salt solution. When paramecia were placed in medium conditioned in this way for 15 days the division-rate was about half that of control cultures. Medium thus conditioned for 30 days actually caused the death of some of the paramecia placed in it. Thus it is seen that bacteria, even a type suitable as food, which accumulate in a culture may have a deleterious effect. In the first experiments of Johnson and Hardin referred to here, most of the bacteria were removed from the cultures each day by the paramecia.

The later studies seem to indicate that a depletion of the food supply is more important as a limiting factor in population growth than are waste products. It seems probable that a depletion of the food supply may have been operating in the earlier investigations where the effects of waste products were postulated as the limiting factor. In most of these earlier works the food consisted of unknown mixed bacteria. As many investigators have found that only a few kinds of bacteria in a mixed wild infusion are suitable as food for a given ciliate, it seems likely in these studies just referred to that a depletion of the suitable bacteria might have caused the early cessation of growth of the populations. This depletion of the suitable food organisms might have been accompanied in some instances by a depressing effect of the unsuitable food organisms remaining in the culture.

The most convincing evidence on this point should be

obtained from studies using the pure culture technique. Phelps (1936) in studies on *Glaucoma piriformis* grown in pure culture on Difco yeast extract and on yeast autolysate found that a population of this ciliate can grow to tremendous densities before the effect of excretory products upon the division-rate is observed. He states that "within wide limits the number of animals at the end of the population is directly proportional to the concentration of nutrient material employed. This is interpreted as indicating that excretory products of the protozoa have no effect either upon the division-rate or upon the final yield until relatively enormous concentrations of animals have been reached" (1,000 times greater concentrations than those reported in earlier experiments).

Lwoff and Roukhelman (1929) found that growth stops in bacteria-free cultures of *Glaucoma piriformis* long before the nutrient materials, as measured by total N, amino N, amide N, and peptone N, are exhausted in the medium. The fact that the supply of N compounds is not greatly decreased before growth stops indicates, according to these workers, that cessation of growth is caused by the accumulation of waste products in the medium. However, there may be another factor, or factors, involved. Such results might be due to the exhaustion of a substance, or substances, present in relatively small amounts in the medium at the start but necessary in small amounts for continued growth of the ciliates. In some recent work on *Colpoda* by Taylor and van Wagtendonk, to be referred to later, the population level attained was found to be proportional to the amount of cytolized bacteria added to the basic yeast extract medium when the same concentration of yeast extract was used in different experiments. Also, in some experiments on the ciliate, *Tetrahymena geleii,* grown in sterile culture on yeast extract, Johnson and Baker (in press) have found that when small amounts of vitamin B_1 are added to cultures which have ceased to increase in numbers further increases occur. Several workers using the pure culture

technique have obtained vastly different maximum populations of the same organism using essentially the same concentration of basic food materials. It may be that these different end results are due to differences in the amount of some growth factor, or of some essential substance present originally only in small amounts, in the different media. It seems likely that further experimentation will show that some of the results which have been attributed to the effects of waste products may be explained in this way.

There is still another aspect to the question of the effects of waste products on a ciliate population. Dimitrowa (1932) reported that growth of *Paramecium caudatum* is accelerated by small amounts of old culture fluid added to fresh cultures. Hall and Loefer in 1938 obtained results which indicate that growth of *Colpidium campylum* in bacteria-free cultures is significantly accelerated by the addition of old culture filtrates to a peptone medium. Kidder (1939) obtained similar results using the same organism. This type of result, a stimulating effect of metabolic products on division-rate, can best be discussed in connection with the next topic—the effects of numbers or density on the population growth.

That increased crowding of organisms or "overcrowding" reduces the rate of increase in the population has long been known to students of population biology. In recent years Allee (1931, 1934 and 1938) and others have presented evidence that a situation of "undercrowding" may obtain in a population, *i.e.,* a population of a few individuals may not show a rate of increase as great as a population somewhat larger but otherwise under the same conditions. In 1921 Robertson reported that two infusoria, either *Enchelys* or *Colpoda,* in the same environment, reproduced from 2.5 to 10 times as fast as did isolated forms. Robertson postulated that a portion of an autocatalyst of growth produced in the nucleus might be lost to the medium at the time of cell division and that two or more organisms would lose less of the autocatalyst

than would one organism in the same amount of medium. This would result in a mutual acceleration of growth or, as he called it, "allelocatalysis."

The controversy raised by Robertson's report is well known. Numerous workers using several different organisms, Cutler and Crump (1923), Greenleaf (1926), Calkins (1926), Myers (1927), Grimwald (1928), Darby (1930), Di Tomo (1932) and Beers (1933), to mention but a few, tried to repeat Robertson's observations but obtained negative results. Yocum (1928) working with *Oxytricha* and Petersen (1929) using *Paramecium* obtained results which were like those of Robertson. Yocum found that single ciliates grew faster in small volumes than in larger volumes of medium, while Petersen found that grouped organisms grew faster than isolated ones in large volumes of medium. When she used small volumes of medium there was no difference. Petersen did not subscribe to Robertson's explanation.

In 1933 Johnson reported that grouped *Oxytricha* reproduce faster than single organisms in the same environment when the concentration of the food organisms (bacteria) was supra-optimal. The grouped *Oxytricha* can control and reduce, during the early stages of the culture, the bacteria more efficiently than can the isolated forms. A similar effect of bacterial crowding has been reported by Chejfec (1929), Barker and Taylor (1931) and McPherson, Smith and Banta (1932). Although these results are similar to those of Robertson, the explanation of them does not support Robertson's theory.

Until two years ago there was little actual support of Robertson's original theory involving a growth autocatalyst, although most workers were agreed that under certain cultural conditions an acceleration of growth might be produced by increasing the starting number of organisms in a culture. Since 1938 the picture has changed. Reich (1938), working with a bacteria-free culture of the soil amoeba, *Mayorella,* obtained a lower division rate with small initial populations than with larger initial populations. He offered no real explanation for his

results. Mast and Pace (1938), as a result of their work on the colorless flagellate, *Chilomonas,* grown in an ammonium-acetate medium, state that this organism produces a growth stimulant which soon accumulates in a culture and becomes a growth inhibitor. They have demonstrated that this substance is heat-labile and, further (1939), that this substance will pass through a cellophane membrane with a pore diameter of 6 mμ.

As was mentioned above, Kidder (1939), has reported results with populations of *Colpidium campylum* grown in bacteria-free peptone-dextrose medium, in the explanation of which he postulated the formation of two substances by the ciliates, one a growth inhibitor which will pass through an asbestos filter and the other a growth accelerator which is absorbed on the filter. In a further study of the effects of old culture medium on the growth of *C. campylum* in sterile cultures, Hall and Loefer (1940) verified their earlier report in 1938, *i.e.,* that the addition of old culture medium, in which the ciliates had been growing, to fresh medium caused an acceleration of growth. In addition, they found that aged medium (3-month-old uninoculated medium) when added to fresh medium produced an acceleration of growth similar to that obtained with the old culture medium in which ciliates had been growing. In commenting on their results they say, "The results might reasonably be attributed to a 'biological conditioning' brought about during growth of the ciliates, if it were not for the fact that aged sterile medium produced comparable effects. Our findings do not demonstrate that the factors producing acceleration of growth are identical in old culture filtrates and in aged sterile medium, and it is possible that they are not the same. On the other hand, it is equally obvious that a 'biological conditioning' can not be invoked as the sole explanation for the accelerating effects of old culture filtrates."

On the basis of these results it would seem that the fresh culture medium was not optimum. In one case the

action of the ciliates made the medium better for their growth, a biological conditioning of an unknown nature, and in the other case, changes, also of an unknown nature, during the period of aging made the medium more suitable. The results obtained with the aged medium do not necessarily detract from the significance of a biological conditioning.

In most investigations of protozoan populations, when good growth is obtained, it appears that an assumption is made, perhaps unconsciously, that the medium is optimum. In many cases this is probably not so. Darby (1930) proposed that the allelocatalytic effect might be a pH effect. He suggested that if the medium is not at the pH optimal for the species and is weakly buffered, since the organisms tend to change the pH, a larger number of initial organisms would change the pH more rapidly and might, then, exhibit a more rapid rate of division. In 1934, Jahn proposed another possible conditioning effect. He said "Since the growth rate is probably affected by the reduction potential, and since the reduction potential of the medium is changed by growth of the protozoa or their bacterial food supply, then it seems as if both positive and negative allelocatalysis could be caused by an adjustment of the medium either toward or away from the optimum for the organism."

In a recent paper, Ludwig and Boost (1939) lend additional support to the idea of a conditioning effect in populations of microorganisms. They have reanalyzed the results of several workers by plotting the relative growth rate against the amount of population produced in the medium. Such a graph gives a picture of the change of the relative rate of growth as the population increases by the addition of new individuals. When Ludwig and Boost analyzed some of the data of Cutler and Crump (1923) on *Colpidium* they obtained the picture shown in Fig. 1. Also in their analysis of some of the data of Gause, Nastukova and Alpatov (1934) they obtained the curves shown in Fig. 2.

This method of analysis indicates that in these experi-

ments there was an initial rise in the relative growth rate and then a decrease. In commenting on this initial rise, Ludwig and Boost attribute it to a beneficial effect of autoexcretory products. They state that many of the protista live in thick populations in nature and that fresh

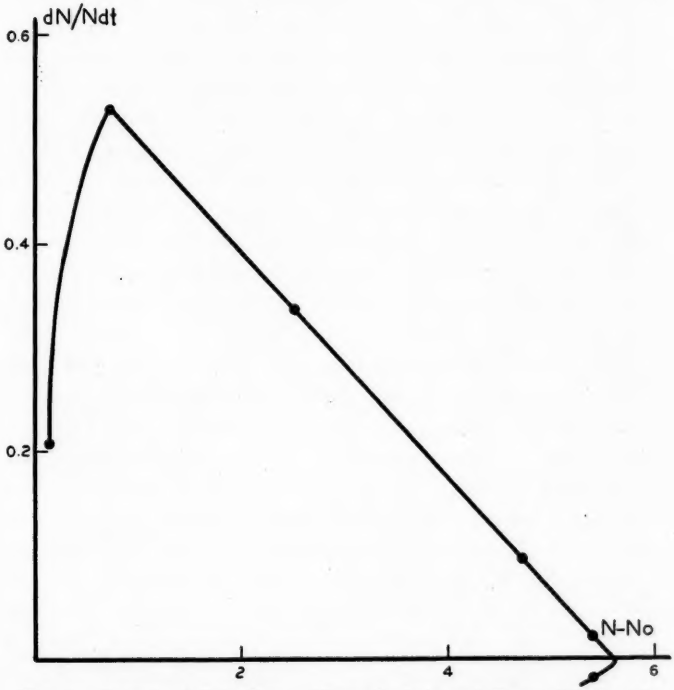

FIG. 1. Growth of *Colpidium*. [From Ludwig and Boost (1939), after Cutler and Crump (1923)].

medium is not necessarily optimal with reference to the excretory products. They speculate that the beneficial working of the excretory products may be through some change in the pH, the redox potential, or some other condition of the medium. In other words, they postulate a conditioning effect.

The status of the problem of the effects of the initial number of ciliates on the rate of growth, after numerous

investigations over a period of several years, seems to be that an increase in initial numbers over the smallest possible initial number may or may not result in an increased rate of division. Some of the recent work on ciliates

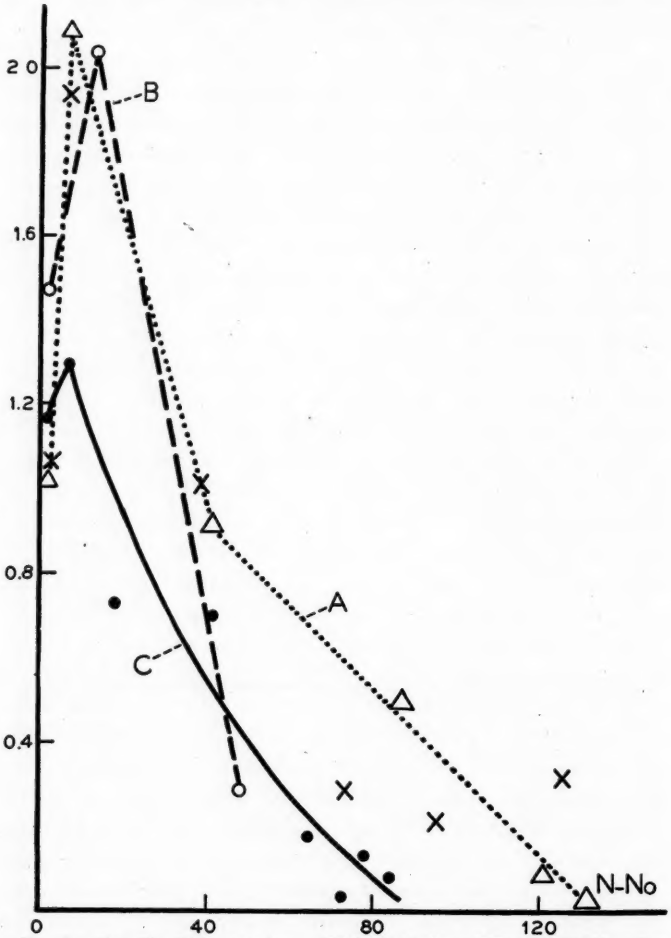

FIG. 2. Growth of *Paramecium*. [From Ludwig and Boost (1939), after Gause, Nastukova and Alpatov (1934)]. Abscissa = number of animals, ordinate = dN/Ndt. Curve A (△) *caudatum* in *caudatum*-medium, B (o) *aurelia* in *caudatum*-medium, C (●) *caudatum* in *aurelia*-medium, D (×, not drawn) *aurelia* in *aurelia*-medium.

suggests that a specific substance favoring growth may be produced. However, whether the acceleration of growth as reported by Kidder (1939) and Hall and Loefer (1940) is due to some specific growth-promoting substance or to some other conditioning effect remains for future work to determine.

In populations of numerous ciliates encystment occurs. In a great many of the studies on this subject, lack of food has been described as the primary cause of encystment. Mast and Ibara (1923), and Penn (1935) regarded metabolic wastes as playing a primary rôle in encystment. In recent studies on *Colpoda,* Taylor and Strickland (1939) report that crowding of the ciliates is of primary importance for the formation of resting cysts in food-free medium. When food is removed from the medium all *C. duodenaria* will encyst. However, unless certain other conditions obtain, resting cysts are not found, but, instead, temporary cysts from which the organisms emerge spontaneously. They say that "in a given volume of this medium the percentage of resting cysts formed depends on the number of organisms present, *i.e.,* the concentration of *C. duodenaria* per cubic centimeter of the food-free medium." Thus they find that in concentrations below 4,400 per cc. the cysts are all temporary, while in all higher concentrations up to 1,950,000 per cc. the percentage of resting cysts formed increases with increasing concentration up to 100 per cent. of resting cysts. They state that this effect may be due to the addition of an encystment-promoting factor to the medium. This type of conditioning of the medium is interesting, along with the other conditioning effects already referred to in other ciliate studies. And here, too, the exact nature of the conditioning remains to be determined.

The effects of metabolic products on populations of ciliates and the effects of initial numbers on the rate of population growth are closely interrelated. The greatest obstacle with which workers in this field have had to contend in obtaining definite and clear-cut results has been the difficulty of attaining standardization and rigid con-

trol of the food or nutritive materials. Maupas (1888) and Jennings (1908) both suggested the need for control of the food in experimental cultures. The first real progress was made by Hargitt and Fray (1917) when they found that *Paramecium* would grow in an infusion containing only *Bacillus subtilis*. In numerous studies since that time it has been shown that for a given species of ciliate some kinds of bacteria are suitable food organisms while others are not.

The recent study of Leslie (1940 a and b) is the most extended study of this kind up to the present. Using over 30 different species of bacteria, carefully standardized as to age and amount used, he tested their suitability as food for *Paramecium multimicronucleatum*. Like other workers he found that some species of bacteria were good food organisms, others were less satisfactory and still others were toxic. He found that different strains of the same species give different results and that the same species might vary in its suitability as food, depending on the age of the culture used. This line of work has led to much better control of the food in ciliate populations, but as long as other species of living organisms are present in such populations it is difficult to determine definitely the cause or causes of the results obtained.

Numerous investigators have tried to obtain growth of ciliates in media containing dead bacteria without success. However, Oehler (1919) using *Colpoda steinii*, E. and M. Chatton (1923) using *Glaucoma scintillans* and D. F. Johnson (1936) using *Glaucoma ficaria* reported growth on dead bacteria. E. and M. Chatton found that growth of *Glaucoma* would occur only after the culture media had previously been acted upon by living bacteria of the species used. Glaser and Coria (1935) obtained growth of two species of *Paramecium* in medium containing either dead yeast or dead bacteria, and, in addition, liver extract and pieces of sterile rabbit kidney. Leslie (1940b) also tried dead bacteria as a source of food for *Paramecium* without success. However, when he used a

ratio of 95 per cent. dead bacteria and 5 per cent. living bacteria he obtained normal growth. By suitable tests he was able to show that the amount of living bacteria present could not account for the growth obtained. On the basis of this Leslie has postulated that living *Pseudomonas fluorescens* furnishes "some growth-promoting substance or food factor for *Paramecium,* the lack of which in suspensions of dead *Ps. fluorescens* may account for its unsuitability."

The obvious solution to the problems involved in the control of the food lies in the pure culture of the ciliates. At the present time seven species of the family *Frontoniidae* have been grown in sterile peptone or yeast autolysate medium in the absence of other organisms. (*Glaucoma piriformis,* Lwoff, 1923; *Glaucoma scintillans,* Hetherington, 1933; *Glaucoma ficaria,* Johnson, 1935; *Colpidium campylum,* Butterfield, 1929; *Colpidium striatum,* Elliott, 1933; *Loxocephalus granulosus,* Hetherington, 1933; and *Leucophrys patula,* Thomas, unpublished). One other ciliate, *Paramecium bursaria,* has been grown by Loefer (1934) in pure culture, but this case differs from the others in that it has associated with it symbiotic green algae, and it has not been possible to grow this species in pure culture when freed of the algae. Using these species various workers have made important contributions with respect to the optimum concentration of the basic food medium and to the effects of adding carbohydrates and fatty acids to the basic medium. Up to the present very little work has been done on the detailed history of ciliate populations in pure culture and the exact nature of their growth curves. The studies of Loefer (1936) on *Paramecium bursaria* and of Phelps (1935, 1936) on *Glaucoma piriformis* indicate, according to these authors, that in general the growth of the ciliates in their pure culture studies follows the general trends observed in populations of bacteria and yeasts. Actually, the greatest deterrent to more rapid progress in this field of investigation has been the failure to obtain pure culture of a greater number of species of ciliates.

Lwoff (1932) who pioneered in pure culture work with his successful culture of *Glaucoma piriformis,* in his treatise on the nutrition of protozoans, concluded, after many unsuccessful attempts to culture other holozoic forms in sterile media, that most of the holozoic forms are obligatory particulate feeders. In discussing the evolution of microorganisms in this treatise Lwoff points out that along with the morphological evolution there has been a physiological evolution involving a successive loss of functions as regards their abilities to utilize different compounds as nutrients. Thus, the simplest protozoans can synthesize their proteins with nitrates as the only source of nitrogen, another group requires ammonium salts, another group amino acids, still another group, to which the ciliate *Glaucoma* belongs, requires peptones, and, finally, the most specialized forms which require more complex particulate food. In a later paper Lwoff (1938) points out that this same idea—a loss of functions—applies with reference to vitamin B_1. Some of the simpler protozoans can synthesize both parts of the vitamin B_1 molecule. Organisms in another group can not synthesize the parts but can fuse them if they are furnished. Still another group must be furnished with the intact molecule of the vitamin. Lwoff says that a veritable state of symbiosis exists in nature between many organisms—one organism producing one part of a necessary substance, another producing the other part of this substance. Also some organisms are completely dependent on others for certain necessary substances.

In a recent paper, Johnson (in press), on the basis of the physiological evolution of the protozoa as outlined by Lwoff and of the findings of Leslie that a small amount of living bacteria enables *Paramecium* to utilize dead bacteria and grow normally, questioned the conclusion of Lwoff that most holozoic forms are obligatory particulate feeders and made this query, "does it not seem plausible that substances comparable to end-products of digestion which can be utilized by other forms may be found, which, along with whatever growth substances may be necessary

in each case, in a sterile liquid medium may support growth of other normally holozoic forms?''

A positive answer to this question has been obtained in the past few months in some significant experiments which should lead the way in the extension of the pure culture method to other holozoic forms. Taylor and van Wagtendonk (in press) have succeeded in the sterile culture of the ciliate, *Colpoda duodenaria.* This ciliate could not be grown on dead bacteria or yeast autolysate medium. Successful culture was obtained by using an extract of dry Brewer's yeast plus a definite amount of cytolyzed bacteria. They found that they could cause the complete breakdown of large quantities of a marine bacterium by adding the bacteria to distilled water. The disintegrated bacteria are essential for the growth of *Colpoda* in the yeast extract. At the present time they have passed their cultures through 8 transfers with no diminution in rate of growth. In these experiments it has been possible to obtain a division-rate as high as any obtained with this organism when fed on living bacteria. As mentioned above, the population level obtained in these experiments is proportional to the amount of cytolyzed bacteria added, within certain limits. This is shown in Fig. 3.

In heat tests on this medium, Taylor and van Wagtendonk have been able to show that heating at 40° C. for a few minutes impairs the growth supporting qualities of the medium and heating at higher temperatures further impairs it. When heated at 70° C. for a few minutes this medium will no longer support the growth of *Colpoda.* Such results may mean that the substance destroyed by heating is an enzyme, or enzymes, which *Colpoda* can not synthesize or obtain from heat-killed bacteria but which it must obtain from living organisms or from organisms killed in such a way that the enzyme or enzymes are not inactivated. This important finding should be heartening to other investigators in the field and should lead to other pure culture achievements.

Population studies are ecological in nature. It seems

at times to some that there is little connection between experiments in test-tubes and bottles and what goes on in nature. This discovery of Taylor and van Wagtendonk with *Colpoda* and that of Lwoff on the vitamin B_1 requirements of different protozoans, each showing a dependency of one organism upon another for some essential substance, indicate how we gradually learn about

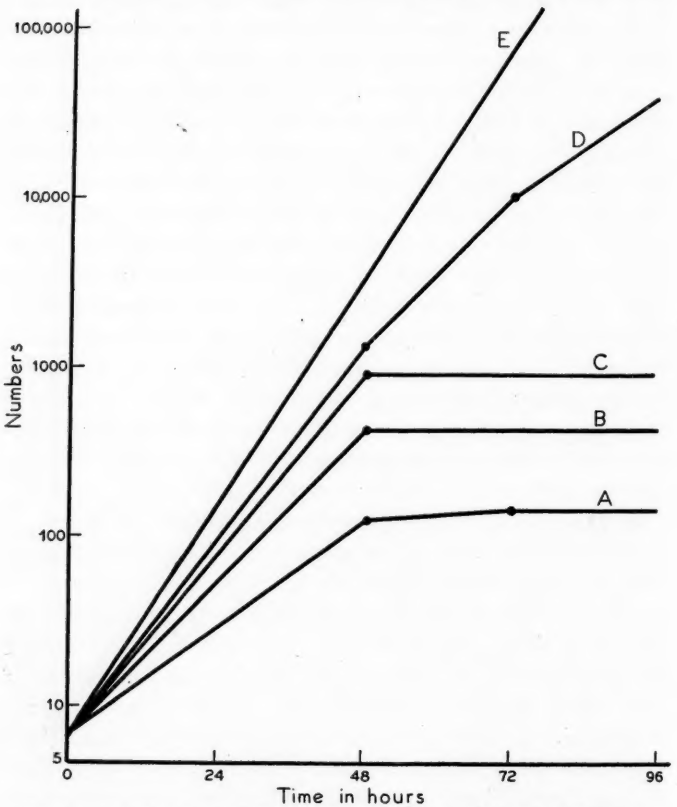

FIG. 3. Growth curves of *Colpoda duodenaria* obtained with different concentrations of bacterial plasmoptyzate. [From Taylor and van Wagtendonk (1941)].

A: 10 gamma per cc of medium.
B: 50 " " " " "
C: 100 " " " " "
D: 500 " " " " "
E: 1000 " " " " "

some of the factors operating in nature. An understanding of such interrelationships as these is possible only through experimentation.

In concluding this review of our knowledge of ciliate populations, it seems in order to suggest that while there are many unsolved problems in the field, there are prospects that many of these will be cleared up in future research. The prospects for further achievements in pure culture work are good. Up to the present time but little work has been done on growth factor requirements of ciliates with the exception of the studies of A. and M. Lwoff (1937) on *Glaucoma* and of Elliott (1939) on *Colpidium,* where, in each case it was shown that the ciliates must be supplied with vitamin B_1. When more is known about growth factor requirements in ciliates and when it is possible to control these substances as well as the basic nutrients in pure culture studies, more specific answers relating to the effects of waste products, of numbers or organisms, of the pH of the medium, of the oxygen tension of the medium, of the CO_2 tension of the medium, of the temperature and of other factors on the nature of population growth, on the duration of the population and on the decline of the population will be possible. In all of this it appears that collaboration of the protozoologist with the chemist should bear fruitful results.

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POPULATIONS OF BLOOD-DWELLING SPECIES

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INFECTIONS WITHIN SINGLE HOSTS

THE development of parasitic protozoan populations within a host varies characteristically in different species of host and with different species of parasite (W. H. Taliaferro, 1941). The total parasite population and its density at any given moment are the result of the interplay, on the one hand, of various factors involving the invasiveness of the parasite, such as reproductive capacity and ability to become acclimated to the specific defensive mechanisms of the host and, on the other hand, of various innate and acquired factors of host resistance or susceptibility. Technical difficulties have limited most of the work on this subject to protozoa which live more or less evenly distributed in the blood stream since they can be extracted from the blood for study without sacrificing the host.

Before discussing the actual infections, some of the difficulties in measuring the rate of reproduction of the parasitic protozoa and a common misconception should be emphasized. While a parasite is within a host, there is no direct method of measuring its reproductive rate comparable in accuracy to the rates determined, for example, in single cell isolation cultures of ciliates. Many authors, in measuring reproductive rates of the parasites, tacitly assume that all the progeny produced by reproduction survive and hence, that the rate of accumulation in the host gives a true rate of reproduction. All measures of the rate of reproduction have to be independent of the number of parasites which perish, however, since in malaria where it has been studied and, probably, in all cases most of the progeny die even under optimum conditions.

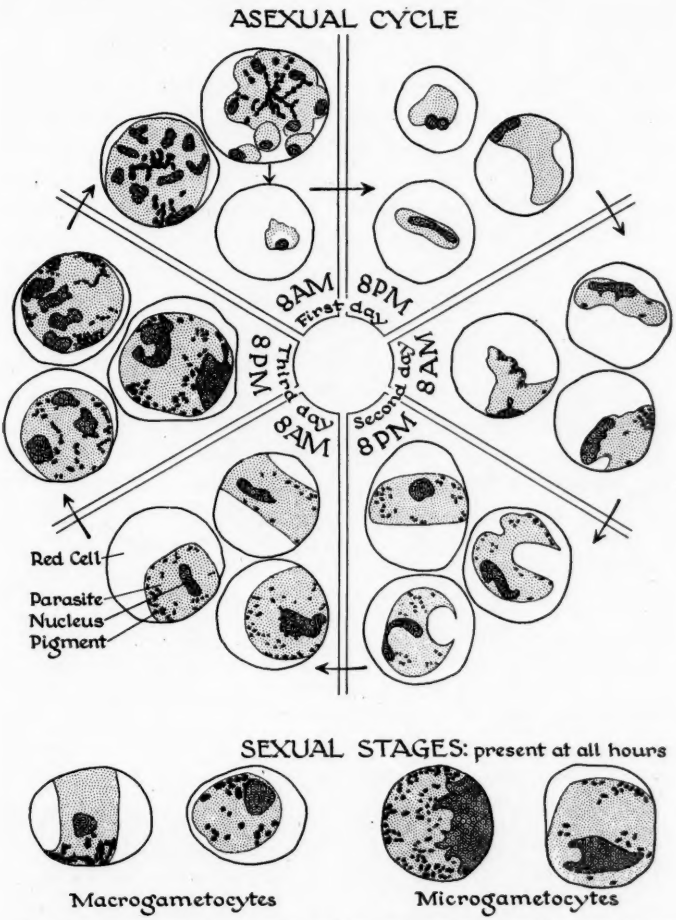

FIG. 1. Representation of the synchronous asexual reproduction of the quartan parasite, *Plasmodium brasilianum*, in Central American monkeys as shown by growth and nuclear changes in the asexual stages at consecutive 12-hour intervals during 3 days. Since the population consists of a single brood, the asexual parasites are approximately uniform in development at each interval. (Infections may consist of two or three broods, each of which segment on different days.) The sexual stages, *i.e.*, gametocytes, which are continuously present in small numbers throughout each 3-day period, are shown for comparison. Modified from W. H. and L. G. Taliaferro 1934.

MALARIA

The synchronous asexual reproduction of plasmodia, as indicated in Fig. 1, permits a direct measure of the rate of reproduction of the parasites, as was first shown by L. G. Taliaferro, 1925. Thus, the length of the asexual cycle gives the time it takes one parasite to mature and produce a given number of progeny. It and the number of merozoites produced by each schizont (suitably corrected for the merozoites which develop into gametocytes) give a measure of the rate of reproduction which is independent of the number which perish. Although temporary delays have been found in the asexual cycle in simian malaria by W. H. and L. G. Taliaferro (1934) and marked changes in the number of merozoites have been found in avian malaria by Boyd (1939), the reproductive rate is maintained at a high level throughout all these infections with no long-continued inhibition, as in the infection with *T.*

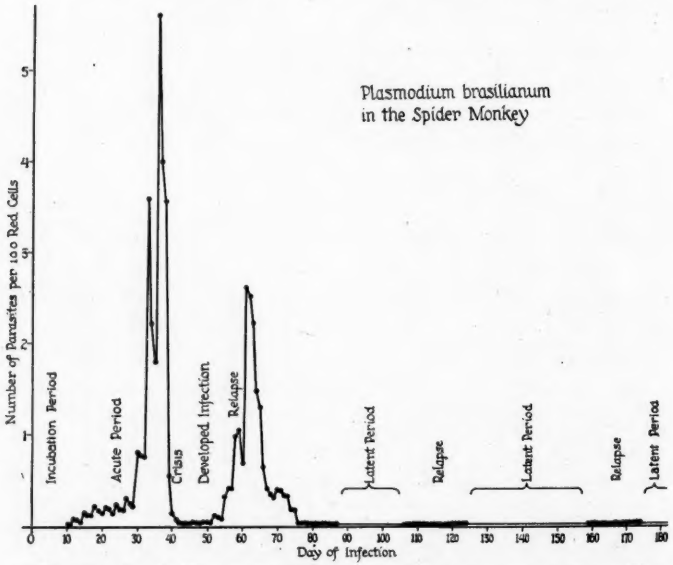

FIG. 2. Graph illustrating the course of an infection of *Plasmodium brasilianum* in a Central American monkey.

lewisi to be described later. In spite of this high rate, the parasites do not generally increase continuously. Thus, as may be seen in Fig. 2, after the incubation period, there is an initial acute rise during which the parasites increase by step-like increments; a crisis, which terminates the initial rise and results in the disappearance of a large majority of the parasites from the blood; a developed infection, during which comparatively few parasites are found; and latency, which may be interrupted at irregular intervals by relapse and which may be terminated by the complete eradication of the infection.

The exact course of the infection varies among the species of plasmodia, but in all infections which have been studied, many parasites perish from the beginning. Thus, to take *Plasmodium brasilianum* in Central American monkeys as an example, each schizont produces an average of 9.5 merozoites every three days throughout the patent infection with the exception of occasional slight delays at the crisis. As only a few of the merozoites develop into gametocytes, a nine-fold increase of parasites would take place every three days if none perished. Actually (Fig. 3), however, even at the beginning of the infection before any acquired immunity supervenes, about 6 of the average 9 progeny fail to infect new red cells during segmentation (Fig. 3, 4/28, 5/1 and 5/4), and of the remaining 3, at least $1\frac{1}{2}$ die during the intracorpuscular development of the schizonts. This gives, therefore, even in the acute rise, a net increase of only about $1\frac{1}{2}$ at each segmentation. During the crisis, which terminates the initial rise and represents the greatly heightened parasiticidal effect of acquired immunity, more parasites perish than are formed by reproduction. Throughout the remainder of the infection, the effect of this acquired immunity varies in intensity. During parts of the developed infection, death of the parasites approximately balances reproduction, and during relapses, the parasiticidal effects of acquired immunity are temporarily relaxed and parasites reaccumulate in the blood.

The death of the parasites, both during natural and acquired immunity, has been demonstrated by Taliaferro and Cannon (1936) to be directly associated with the ingestion of parasites by the macrophages of the spleen,

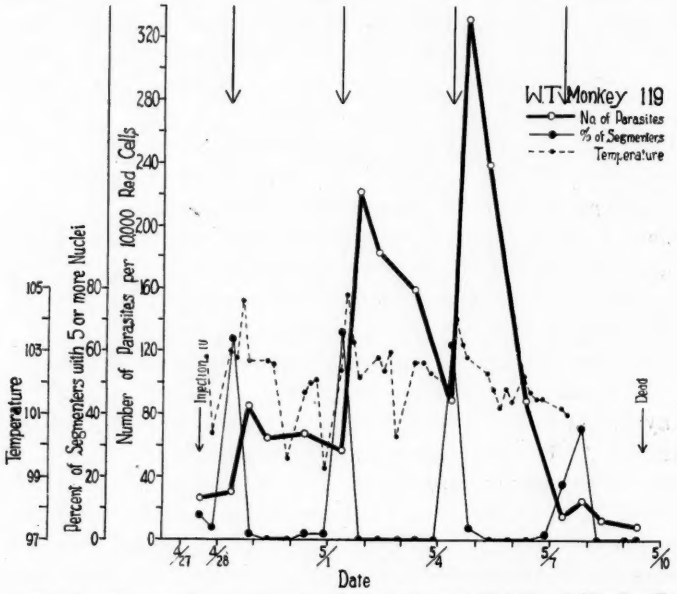

FIG. 3. Changes in the number of parasites per 10,000 red blood cells and the asexual reproductive cycle (measure of the rate of reproduction) during the acute rise and crisis of an infection of *Plasmodium brasilianum* in a Central American monkey. There is no pronounced or long-continued inhibition of reproduction, but both natural and acquired parasiticidal effects are pronounced as indicated by the following: Constancy of the rate of reproduction is indicated by the fairly uniform development of 9 merozoites from each parent schizont (see text) regularly every 3 days during the acute rise of the infection (see the peaks in the per cent. of schizonts with 5 or more nuclei and the peaks in temperature). The rate is temporarily less at the crisis. A natural parasiticidal immunity is operative during the acute rise of the infection, as evidenced by the death of parasites during the segmentation periods on 4/28, 5/1 and 5/4 when the increase in parasites is never equal to the number of progeny produced and during the intersegmentation development from 4/29 through 4/30 and 5/2 through 5/3 when the parasites decrease in number. An acquired parasiticidal immunity is operative at the crisis as evidenced by the increased death of parasites on 5/6. From W. H. Taliaferro 1932.

liver and bone marrow. These organs are strategically placed to remove material from the blood stream. Recently, Gingrich (1941) has found that phagocytosis of the parasites during natural immunity in avian infections is probably not a primary process, but represents the ingestion of impaired organisms. In other words, the macrophages are scavengers and remove parasites which are moribund due, possibly, to an innate death rate of the parasite (Hartman, 1927), to injury of the host red cell (Hewitt, 1938) or to the unsuitability of the host as a culture medium. Gingrich further found that phagocytosis becomes a primary process after acquired immunity is initiated. It actively removes the invaders and can be significantly reduced by so-called blockade, *i.e.*, by injecting foreign material which the macrophages ingest. This increased active phagocytosis of acquired immunity is probably aided by antibodies, such as opsonins, which make the organisms more readily ingested by the phagocytes (see Coggeshall, 1940). The antibodies are probably secreted by the same macrophages that ingest the organisms.

The recently described exo-erythrocytic stages in malaria, which may markedly modify some of the interpretations given in the foregoing account, have been purposely omitted. Their nature and occurrence is too imperfectly known to permit profitable discussion.

The Trypanosome Infections

The trypanosomes exhibit no synchronous reproduction, *viz.*, all kinds of dividing and growth forms may be found in any given sample in which reproduction is occurring. So far, therefore, the methods of measuring their rate of reproduction have given comparative rather than absolute values such as have been obtained among the plasmodia. Two basic measures have been used. The simplest method involves the direct microscopical examination of stained blood films for cell division of the parasites by noting the percentage of division forms (Robertson, 1912; W. H. Taliaferro and Pavlinova, 1936).

The second method (W. H. and L. G. Taliaferro, 1922) necessitates ascertaining the coefficient of variation for some selected measure of size because, within limits, size varies directly with the rate of reproduction (Fig. 4). In spite of certain theoretical objections, these methods give a valid measure of the comparative rate of reproduction which is independent of the organisms that perish (see discussion in W. H. Taliaferro, 1941).

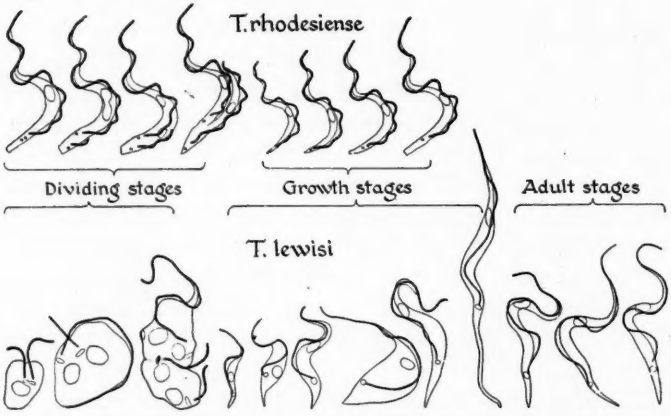

FIG. 4. Variability in size associated with division and growth in *Trypanosoma rhodesiense* and *T. lewisi*. In *T. lewisi*, in which reproduction may be inhibited, adult (resting) stages can be found and are remarkably uniform in size and structure. In *T. rhodesiense*, in which reproduction is not inhibited, adult stages can not be accurately determined. The increase in variability in total length associated with division and growth is used in Figs. 5 and 7 to give a comparative measure of the rate of reproduction. Modified from W. H. and L. G. Taliaferro, 1922, from drawings by F. A. Coventry, and from W. H. Taliaferro 1923.

THE PATHOGENIC TRYPANOSOMES IN THE MOUSE

The simplest type of infection is found when the so-called pathogenic trypanosomes (*e.g., Trypanosoma gambiense, T. brucei, T. evansi* and *T. equiperdum*) are grown in the mouse (Fig. 5A). The rate of reproduction of these forms, as ascertained by the previously mentioned methods, is maintained at a high and fairly uniform rate throughout the infection. In addition, once the infec-

tion becomes patent, the organisms increase according to a geometrical progression until the death of the host. As there is no change in either the rate of reproduction or the rate of accumulation, there can be no appreciable *acquired* resistance producing either an inhibition of reproduction or marked death of the parasites. Nothing can be said

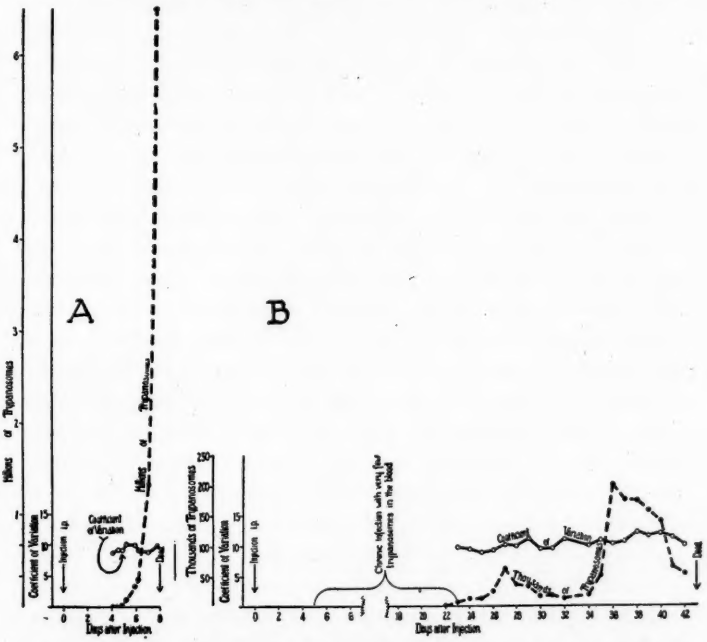

FIG 5. A. Number of parasites per cmm of blood and the coefficient of variation for total length (comparative measure of the rate of reproduction) throughout the course of an infection of *Trypanosoma rhodesiense* in a mouse. There is no appreciable inhibition of reproduction or parasiticidal immunity acquired during the infection because the rate of reproduction is maintained unchanged and the parasites accumulate in a geometrical progression. B. Number of parasites per cmm of blood and the coefficient of variation for total length (comparative measure of the rate of reproduction) throughout the course of an infection of *Trypanosoma rhodesiense* in a guinea pig. The rate of reproduction is maintained unchanged throughout the latter part of the infection, but acquired parasiticidal immunity is indicated by the two periods of decreasing numbers of parasites and is probably operative during the early part of the infection. From W. H. and L. G. Taliaferro 1922.

about a possible natural immunity or suitability of the normal nonimmune host as a culture medium for the trypanosomes. Thus, it is not known whether reproduction is maximal or whether a constant number of the progeny perish as they do in malaria.

THE PATHOGENIC TRYPANOSOMES IN THE GUINEA PIG

When the same pathogenic trypanosomes considered in the preceding section are grown in guinea pigs and certain other animals, the first part of the resulting patent infection may be similar to that occurring in mice, *i.e.*, there is a high and more or less uniform rate of reproduction and a uniform geometrical increase of the parasites in the blood. Just as in the infections in mice, experimental data do not permit definite conclusions regarding possible natural or innate antiparasitic effects during this time. The acute infection, instead of leading directly to death as in the mouse, however, is profoundly modified by an acquired resistance of a parasiticidal type. Thus, the basic rate of reproduction remains high and more or less uniform, but the organisms, which accumulate in the blood during the acute rise, are suddenly swept from the blood in varying numbers and, at intervals thereafter, relapses and crises follow until the animal dies (Fig. 5B).

Suitable experimentation by a number of investigators, notably by Schilling, Franke, Ehrlich and his coworkers, Rodet and Vallet, Massaglia, Levaditi and his coworkers, and Ritz (W. H. Taliaferro, 1926), has shown the following: The wholesale death of trypanosomes at each crisis is the result of a typical antibody, a trypanolysin, which occurs in the blood, but which rarely kills all the organisms. The few trypanosomes that remain become resistant to this antibody, and, as this resistance is inherited for many asexual generations and as the rate of reproduction remains high, the organisms repopulate the blood stream and, thereby, produce a relapse. As the trypanosomes become antibody-resistant, the specific chemical in them, *i.e.*, the antigen which stimulates the production of

the lysin, also becomes altered. This change in the lysin-stimulating antigen is important as it may constitute the basis of antibody resistance by removing the chemical in the parasite which reacts specifically with the lysin and by supplying a new antigen in the relapse population. The new antigen, in turn, however, may stimulate the production of a new lysin effective in removing most of the relapse parasites from the blood stream (Fig. 6). In

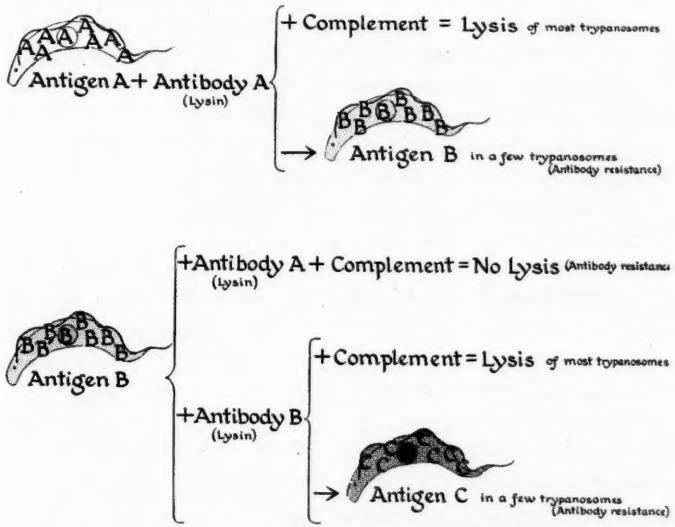

FIG. 6. Diagram of the probable relationship of antigenic changes to antibody resistance of the pathogenic trypanosomes. Each lysin when produced by the host kills a majority of the homologous trypanosomes, but a few of the trypanosomes escape its action by becoming changed antigenically.

fact, this process is usually repeated several times before the host actually dies. A large number of antigenic variations and specific antibody resistances can be acquired within a clone of trypanosomes. In passing, it should be noted that the isolation and maintenance of the antigenic variants is done in mice in which no antibodies are produced normally and hence in which the clones are not split into antigenic variants.

The acquisition of antibody resistance with a con-

comitant antigenic change is one of several characters all of which seem to be environmentally induced in trypanosomes and may be inherited for many asexual generations. Another is their resistance to drugs in ordinary curative doses. Acclimatization to certain of the dyes belonging to the pyronin, acridin and oxazin group may result in the loss of a cell organelle, the parabasal body.

The underlying mechanism is probably the same as when free-living species become acclimatized to various chemicals. Whether or not such changes are actual mutations due to genic changes or are Dauermodifikationen due to cytoplasmic changes, as maintained by Jollos, is unsettled (see W. H. Taliaferro and Huff, 1940) and may possibly only be settled provided the phenomena can be studied in forms which can be followed individually in sexual crosses, as Sonneborn and Lynch (1934) have done in *Paramecium*. Irrespective of the genetic nature of these resistances, the following should be noted: The acquisition of antibody or drug resistance is generally associated with the death of a large proportion of the original population. Several investigators (for a review and discussion, the reader is referred to W. H. Taliaferro, 1926; W. H. Taliaferro and Huff, 1940) believe that the resulting selection is of crucial importance, but, although this contention may be true, it is interesting to point out that it acts unusually quickly and is effective in a single clone. The resistance may be inherited for as many as 400 mouse-passages, which represent countless asexual generations of the parasite. It may be quickly lost and is generally lost when a new antibody or similar adverse environmental factor acts on the population. In some cases, however, as in the resistance of *T. brucei* to arsenicals, it can persist after two passages through the invertebrate host. These apparently environmentally induced modifications are probably of considerable importance in the survival and possibly in the evolution of the species. Thus, they certainly enable the parasite to overcome some of the defensive mechanisms of the host and, at times, may be persistent enough to be acted upon

by natural selection. It is barely possible that the change in antigenic composition following the action of a specific antibody may represent a specifically induced change in the protein or carbohydrate structure of certain genes.

The Nonpathogenic Trypanosomes, Especially *Trypanosoma lewisi* in the Rat

The microscopical studies of early workers, notably Rabinowitsch and Kempner and Laveran and Mesnil, convinced them that *T. lewisi* was peculiar in that its reproduction was limited to the first week or ten days of the infection in the rat. Later studies by the author (1924) indicated that this inhibition was associated with a peculiar antibody, *i.e.*, ablastin, which inhibits the cell division of the organisms, but does not kill them. Thus, trypanosomes may live in the presence of ablastin for many months in an active, nonreproducing state and are fully infective to other rats. Similar ablastins have also been demonstrated in certain other nonpathogenic trypanosomes of the *T. lewisi* group. In addition to the development of ablastin in infections of *T. lewisi*, specific parasiticidal effects which kill a large number of the parasites are acquired, generally at about the tenth day of the infection and at intervals thereafter until the patent infection is terminated. These parasiticidal effects have been demonstrated to be due to specific trypanolysins, similar to the ones produced in the pathogenic infections, and are possibly aided by phagocytosis of opsonized parasites, as in malaria. Although the parasites can apparently become acclimated to these parasiticidal antibodies, relapses do not occur as in the pathogenic infections because the trypanosomes are not reproducing (see adult stages in Fig. 4) and can not repopulate the blood (Fig. 7). Occasionally, however, a relapse may occur because the production of ablastin is lowered in the rat due to the simultaneous occurrence of certain other infections or various experimental procedures, such as splenectomy or blockade of the macrophages along the

blood stream with India ink or other foreign material (W. W. Taliaferro, 1941).

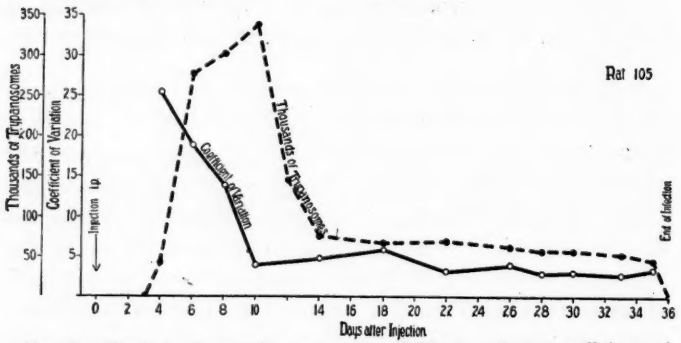

FIG. 7. Number of parasites per cmm of blood and the coefficient of variation for total length (comparative measure of the rate of reproduction) throughout the course of an infection of *Trypanosoma lewisi* in a rat. Both reproduction-inhibiting and parasiticidal effects are acquired during the infection. Thus, reproduction of the parasites is inhibited by about the tenth day and large numbers of parasites are killed beginning on the tenth day and at the end of the infection. From W. H. and L. G. Taliaferro 1922.

RÉSUMÉ

The preceding discussion consists essentially of illustrative examples of the environmental conditions, *i.e.*, what the immunologist terms the natural and acquired immunities of the host, which influence the populations of the hematozoa within single hosts. In malaria, even under the best of conditions, *i.e.*, in a highly susceptible host before the onset of acquired immunity, the host is far from an ideal medium because a great majority of the parasites perish (innate or natural immunity). In infections with the pathogenic trypanosomes in mice, no data are available on the presence of such natural immunity, but the parasites are generally unaffected by factors of acquired immunity. In both malaria and infections with the pathogenic trypanosomes in the guinea pig, the parasites maintain a high rate of reproduction, but periodically large numbers of them are killed by parasiticidal factors of acquired immunity. In malaria, these parasiticidal factors, *i.e.*, opsonins and phagocytes, may keep the numbers

of an actively reproducing population at a low level over a long period (developed infection and probable latency), but may be temporarily removed and, thus, allow the parasites to reaccumulate (relapse). In infections with the pathogenic trypanosomes, the parasiticidal factors, *i.e.*, lysins which produce each decrease in parasites, remain in the host, but the parasites, because they possess the ability to become hereditarily resistant to them, can reaccumulate in the presence of lysins which were fatal to their original progenitors. In infections of *T. lewisi* in the rat and related trypanosomiases, the host, in addition to developing parasiticidal antibodies, forms an antibody, *i.e.*, ablastin, which completely inhibits the reproduction of the parasite. Therefore, even when the parasite becomes adapted to the parasiticidal mechanisms of the host, there can be no relapse because the reproduction-inhibiting antibody is still operative.

Infections within Host Communities

Besides populations in single hosts, populations of parasites might be considered from the view-point of entire host communities. Some years ago, Ross developed a system of differential equations to represent, under certain conditions, the propagation of malaria within a community. The Ross equations have been extended and developed by others, in particular by Lotka (1923). Students of evolution have used these equations to express the struggle for existence. From the standpoint of the present discussion, it is obvious that the growth of malaria and the attainment of malaria equilibria among human and mosquito populations as given by the Ross equations can be used to measure the total parasite population in a community of vertebrate and invertebrate hosts. Under natural conditions, almost all the infections that have been considered in this chapter are transmitted by insects. Hence, the Ross equations could be suitably modified for each, but as far as they have been developed, they apply only to idealized conditions. Some future mathematician may possibly develop the analysis taking into account such

factors as lethality and length of infection, different types of acquired immunity and the ability of the parasites to adapt themselves to various immune mechanisms of the host. Lotka, however, has pointed out in the case of malaria that further analysis along more realistic lines may lead to considerable mathematical difficulties.

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[1] This consists of reviews and a few papers on original work which is particularly stressed in the text.

INTEGRATION OF PROBLEMS CONCERNING PROTOZOAN POPULATIONS WITH THOSE OF GENERAL BIOLOGY[1]

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A LIFE-LONG student of problems associated with the Protozoa remarked recently that the results obtained from research with Protozoa are mainly applicable to protozoan problems only. Questions of sex aside, he continued, such studies contribute little to our knowledge of the biology of the Metazoa. Perhaps on reflection this biologist might decide to add odds and ends of other reservations. He might, for example, remember that studies on the mechanics of movement of Amoeba illuminate all studies on amoeboid movement wherever found.

The statement with which I began, even with further reservations, represents the modern reaction against a former belief (implicit rather than explicit) that, broadly speaking, Protozoa are relatively simple, generalized organisms. Fifty years ago, students of behavior in particular seem to have thought that in the protozoans behavior might be found in its simplest terms. We now appreciate that the Protozoa are not necessarily primitive animals. In their long evolutionary history they have become efficient Protozoa which are not necessarily persistent bearers of generalized protoplasm.

This growing acceptance of the organismal as opposed to the cellular concept regarding protozoans has, however, increased their usefulness in comparative population studies. To be sure, Protozoa present certain specialized population problems, some of which have been

[1] This discussion was presented as the concluding paper in a symposium on protozoan populations and should be read in connection with the program that preceded it. The other papers in the symposium are starred in the bibliography. I am indebted to Miss Ruth Merwin, Mr. Asher Finkel and to Drs. A. E. Emerson, R. H. Hall, W. H. Johnson, Thomas Park and H. S. Smith for reading the manuscript critically.

elaborated by preceding speakers in the present symposium. Problems associated with bacterial feeding furnish an example of such special relations; but even these have broader applications since, methods of control aside, it makes little difference to the student of populations whether his beasts feed on microscopic fodder or the kind that comes in bales. There may be hay and weeds in both.

The culture of any given experimental animal presents special technical difficulties; a certain percentage of the results obtained are of interest primarily to students of the particular species under investigation. But study of cultures of any species should also give general results which are applicable to many different types of organisms. My task in the present program is to emphasize certain of the larger implications which grow from the consideration of protozoan populations.

Along with other animals, protozoans are affected by two main classes of factors: (1) There are those conditions which are produced by the physical environment (*cf.* Hutchinson, 1941). These include such matters, for example, as temperature, pH and the size of the effective environment. Environmental influences can be divided into those such as (a) climate, over which the protozoan assemblage may have no effective control, and (b) matters of environmental inadequacy, certain types of which can be affected readily by processes of population physiology. For example, there may be mass fixation of some toxic material either by adsorption or by combination with metabolic products. This represents one phase of biological conditioning of the environment, a process that usually proceeds more efficiently in the presence of fairly large numbers of organisms. (2) The second class of factors which affect populations is composed of those which arise from the activities of the population itself. These include, for example, (a) the accumulations of excretions and secretions and their decomposition products; (b) the effects of food and oxygen consumption, and (c) behavior interactions.

The environment plays an important rôle even with those relations which arise primarily within the population. The size of the effective environment regulates the concentration of metabolic products. It controls, to some extent, the total amount of food and oxygen to which the organisms are exposed, and affects many phases of crowding. The larger the effective environment, the greater the dilution of toxic material, the more weakened certain types of physical disturbances will be at the periphery and the larger the quantity of missing elements which the population may have to supply in order to establish optimum or even adequate living conditions.[2] It follows that the residue of effects which are purely internal to the population is small and consists mainly of those cases in which mutual attraction or repulsion brings about interactions between individuals. Even these interactions will not often occur unless the available space is small enough so that the animals are obliged to live within reacting range of each other.

Environmental agencies, climate for example, may completely control the existence of the population. Such agencies are more important in nature than in experimental populations since, for obvious reasons, environmental extremes are normally avoided under laboratory conditions.

The entomologists have carried the analysis of the control of population size, and of other aspects of population physiology, a step further. Smith (1935, 1939) and Bodenheimer (1938), among others, have discussed these

[2] If Darby (1929, 1930) is correct in his conclusion that ciliates produce a significant change in the pH of their culture media apart from the effects caused by associated bacteria, such adjustment of the pH is a part of the work done by protozoans which is affected by the size of their immediate environment. However, Garner (1934) found that Paramecium did not appreciably change the pH in his cultures. Pending further evidence, we must reserve judgment concerning the extent to which the ciliates themselves change the pH of their environment. This is an important point since much of Robertson's phenomenon (often referred to as allelocatalysis) and certain phases of mass protection of Paramecium from heat could be explained fairly simply if Darby is correct.

problems in terms of the size of the populations themselves, in addition to considering whether control rests within the biota or in its environment. They are much interested in that question, too, and a brief consideration of their ideas will be helpful.

Essentially these entomologists recognize two sets of controlling influences: those which depend on population density and those which are independent of density. The so-called density-independent factors kill off the same percentage of the organisms in dense and in sparse populations. Generalized climatic agents work in this fashion. Even such climatic forces, however, may become density-dependent if the environment lacks a suitable number of protective niches during periods of special climatic stress.

Density-dependent factors are said by Smith to be those which produce an increasingly high percentage of elimination with increasing density of the basic population. For insects, parasites are such density-dependent factors and are of great importance in the control of insect numbers. With parasites, immunity reactions aside, the larger the host population, the larger the percentage of it which is likely to be attacked and destroyed. With insects and with protozoans and with many other animals, intra-specific population pressure acts in this manner.[3] It would be interesting to know how the populations of protozoan parasites (Taliaferro, 1941) would fit into this scheme.

There is another type of density-dependent factor which, while recognized by these entomologists, is dismissed by certain of them as of no importance in population control. I refer to those eliminating influences which take a decreasing percentage of the individuals present as the population increases. Population reduc-

[3] Another direct density-dependent effect in protozoan populations is reported by Taylor and Strickland (1934). They find that Colpoda encyst in increasing percentages, the more dense the population. The encysted organisms are removed from competition for the limited food supply and to that extent parallel the more permanent eliminations by parasitism or predation.

tions caused by predation frequently follow this pattern. The difference between control of numbers by parasites and by predators may be chiefly a matter of their relative speed of reproduction, or of their respective power of discovery of suitable prey.

A part of the inter-relationships between these different control agencies is indicated in Fig. 1, which presents an idealized situation in a population that is changing in

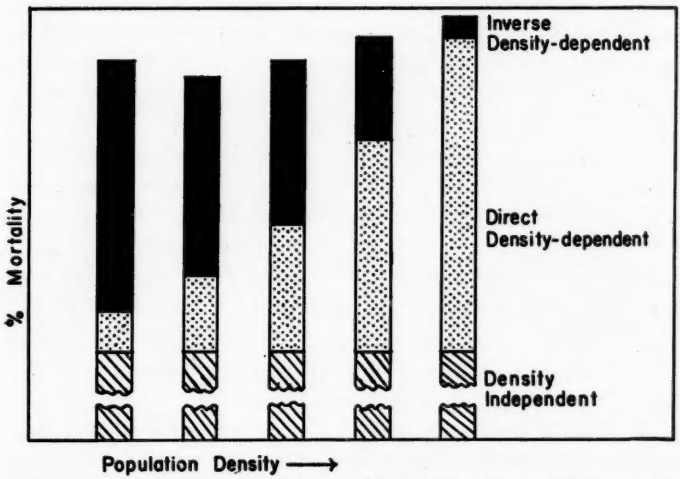

FIG. 1. A schematic presentation of the relation between different types of population control. Detailed explanations are given in the text.

size. The vertical axis gives the percentage of mortality and the horizontal axis shows population density. The density-independent eliminators are shown taking a steady toll which has been arbitrarily set. The effect of *direct* density-dependent factors, which remove a small percentage when the population is low in numbers and which become increasingly effective as numbers increase, is shown by the stippled blocks. The upper solid blocks which decrease with increasing density show the effect of the *inverse* density-dependent eliminators which kill off many more individuals in the higher concentrations, although the total percentage which they destroy is decreased. The residue represents survival.

It seems obvious, despite the objections of Bodenheimer (1938), that the direct density-dependent factors are the most potent means of control when the population tends to increase beyond its usual limits. Neither of the others can, however, be lightly dismissed, for, as Smith (1935) suggested in another connection, the elimination of even a small percentage during times of crisis may become of great importance in regulating final population size.

The inverse density-dependent factors have some interesting theoretical implications since with them, as the numbers present increase, there comes an increasing chance of survival for any given individual. The direct density-dependent factors are those whose operation led earlier observers to conclude that all crowding is harmful; that is, has negative survival value. The inverse density-dependent influences give one illustration of a more recent emphasis: crowding may have a positive survival value for some or all of the individuals. In so far as they operate, the protection furnished by numbers is shown by the decrease in percentage eliminated by the inverse density-dependent agencies.

It may help at this point to consider some of the simpler conditions that actually occur in early stages of drop cultures of protozoans. First, take the situation in which one bacteria-free protozoan is introduced into a drop of bacteriologically sterile medium. There is one organism in a small environment which represents a physicochemical habitat reduced to simplest terms. In the language of certain ecologists, when an organism is affected by its habitat we have an *action,* and when it influences its habitat, the process is said to be a *reaction.* The whole represents an ecological action-system. As regards the continued existence or the well-being of the protozoan, the action of the habitat on the introduced organism or its reaction on the culture drop, or both, may be neutral, beneficial or harmful.

If a second active protozoan is placed in such a simpli-

fied microcosm, the resulting changes in the ecological action system consist principally of the direct or indirect effects of one animal on the other. Such direct effects are called coactions by certain ecologists.[4] Some of the many possible types of interactions are illustrated in Fig. 2, in which B, C, E and F represent coactions in part

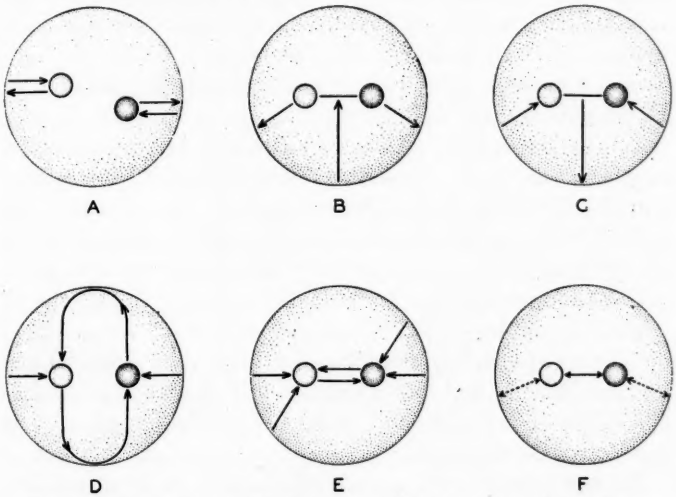

FIG. 2. Some of the possible types of ecological action systems when two organisms are living in a common environment.

and D is difficult to separate from them except by formal definition. Coactions may be essentially neutral, beneficial or harmful for either or both of the protozoans concerned. Actually complete neutrality is very rarely encountered in such a simple ecological action system and hence needs no further consideration.

Any coaction may have beneficial or harmful aspects regardless of the combined end effect; and in the case of the entire community, it is essential to appraise the survival value of given coactions on the community as a whole. Mating is one kind of coaction in which the habi-

[4] Dr. Thomas Park's forthcoming discussion in the *Quarterly Review of Biology* for 1941 should be consulted in this connection.

tat may play a passive rôle. This is then an inter-individual coaction in pure form which may be beneficial to the race regardless of the effect upon the individuals themselves. Or the two protozoans we are considering may coact with each other so violently that both are obviously injured in the process. For example, when the cut surfaces of two pseudopodia from the same clone of *Arcella* but which have been cultured under different conditions or are distantly related within the clone are brought in contact, both may be shattered by the coaction (Reynolds, 1924).

In their reaction on the habitat, the two protozoans may fix some toxic material more efficiently than either can do alone, or may otherwise jointly condition their habitat more effectively in one of several ways. Certain of the results of such conditioning are often discussed under the heading of "Robertson's effect," or loosely as "allelocatalysis." This is an example of a basic positive survival value which involves the habitat and may have various manifestations some of which have been discussed by Drs. Hall and Johnson in this issue of the AMERICAN NATURALIST.

As has been shown in their discussion, such effects are more wide-spread than was suspected even fairly recently. Within the last three years the discoveries of Mast and Pace (1938), of Reich (1938), of Kidder (1939) and of Hall and Loefer (1940), together with the recalculations of Ludwig and Boost (1939) make this quite plain. The explanation may be almost as diagrammatically simple as the system I have been presenting. In its whole ramifications, however, it is probably much more complicated and involves the bacterial population as well as the coacting protozoans.

It will be well to pause here to get a matter of terminology straightened out. Clements and Shelford in their important book, "Bio-ecology" (1939), regard helpful coactions as evidence of *cooperation* and harmful coactions as evidence of what they call *disoperation.* With

lower forms and with poorly integrated ecological systems, the effects are wholly non-conscious. This is a consideration which does not affect the fundamental nature of the resulting disoperation or cooperation. It is essential to remember that, possible neutral effects aside, we are dealing with a population system in which the survival values present only one essential dichotomy into beneficial (cooperative) and harmful (disoperative) effects.

Competition furnishes a special phase of both cooperation and disoperation. Clements and Shelford (p. 160) define it as follows: "In general, competition is to be distinguished from all other coactions by the test of a common demand on a limited supply. This criterion applies even to the combat between two males for the same mate." They also point out (p. 160) that there is an element of competition in certain types of disoperation.

A return to the simplified ecological action system may help evaluate the relation between the concepts of competition, cooperation and disoperation. Two protozoans in a drop culture with limited salt content may be forced to compete for the inadequate amount of a necessary ion which may be present. The competition may result in the stunting of both. Or, more obviously, disoperative competition in such a culture may result from a limited nutritive supply. An example of cooperative competition at the same level of organization is harder come by unless Richards (1941) is right that Protozoa swim about more in large drops than in small and so use more energy and hence divide less rapidly.

An interesting analogy with the activity of particles in a wholly physical system may be suggested at this point. Some will recall van der Waal's modification of Boyle's familiar gas law in which it is recognized that the behavior of molecules of gases is different when the molecules are far apart at low pressures from that shown when pressures are higher. Under the more crowded

conditions, the molecules exert a mutual attraction which changes their activity sufficiently that Boyle's original law no longer describes their behavior (Getman and Daniels, 1931, p. 13).

To the extent that Richards is correct regarding the effect of size of drop upon the amount of movement in Protozoa, the observed effect can be interpreted as meaning that because of competition for space in a small volume of medium, two organisms have their locomotory activities slowed down and thereby save energy which is used in more rapid growth and division. Such a competition for space results in a form of non-conscious cooperation.

At a higher level of integration, when competition results in a dominance-subordination pattern, whether of plant stratification in a forest, or in the peck-order type of social organization of flocks of birds and other social groups, beneficial results for the whole community can be demonstrated. Competition here becomes beneficial in its final effects. Such dominance-subordination patterns of behavior are not to be expected in the type of sociality that exists among the Protozoa. The illustrations used again show that there are two distinct types of coaction which are antithetic in their effects; the harmful (disoperative) and the beneficial (cooperative) phases. Competition can have a foot in both camps.

In attempting any penetrating assay of the cooperative and disoperative aspects of competition, and frequently in distinguishing fundamental cooperation from disoperation regardless of competition, we must recognize that there may be short-run and long-run results that are not necessarily similar. Robertson's effect, for example, may allow a properly seeded culture to establish itself somewhat more rapidly and to overcome the handicap of the presence of too many bacteria (Johnson, 1933). Clearly this is beneficial for the short run. Even so, the end result may be that the protozoan population reaches its asymptote sooner and the onset of disoperative effects

of overcrowding is hastened. It is easy to visualize conditions in which the rapid building up of a sizable population is beneficial to an emigrating species and may indeed be the determining factor in its successful establishment; this holds true despite the possible long-run disadvantage of an overly high rate of reproduction when the food supply is limited.

The disoperation brought on by overcrowding may serve to eliminate those individuals which are unable to withstand the crowded conditions under which many populations are forced to live. The long-run effects of such elimination depend in part on whether other and more desirable traits are lost in the selection of a crowd-resistant strain. This means, all things considered, that whether the final results of such disoperation are of advantage or of disadvantage to the race depends frequently on the criteria of values which may be used. This is a problem many of the implications of which are recognized by any thoughtful biologist. Normally he side-steps their consideration by restricting attention to those values whose effects can be measured in terms of survival values alone.

If we restrict attention to matters of population density, it can be demonstrated readily that the short-run survival of many protozoan populations and of those of other animals can be summarized by the curve given in Fig. 3. In this curve, percentage of survival is shown on the vertical axis and population density, increasing from left to right, is indicated on the horizontal axis. Protozoan survival values so related to density have been reported, among others, by Sweet (1939) for resistance to many unfavorable environmental conditions; and earlier by Drzewina and Bohn (1921) for certain toxic reagents. Similar relations have been found as regards survival and many other biological processes among various groups of organisms throughout the animal kingdom (*cf.* Allee, 1931, 1934, 1938).

Evidence is steadily accumulating concerning the

reality and the biological significance of phenomena related to undercrowding which are represented by the left-hand limb of the curve (b_1) as distinguished from the long-recognized and important phenomena associated with overcrowding which are summarized by the right-hand limb (b_2).

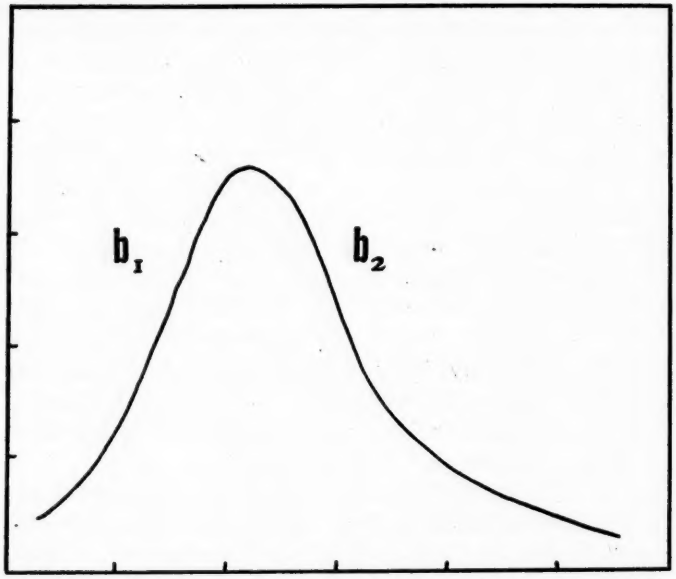

FIG. 3. In certain phases of population physiology the optimal population is intermediate in size.

The implications of overcrowding have been well emphasized by others and, aside from again stressing their importance, I shall not consider them further. The suggestions that flow from the phenomena of undercrowding have been less fully discussed, and I may be permitted to summarize certain phases very briefly. In my haste I take the risk of seeming to be dogmatic. I have developed similar ideas more carefully and at greater length in other publications (*cf.* Allee, 1940).

The existence of undercrowding and the consequent likelihood of the existence of an optimal population which

is larger than the minimum necessary for reproduction, indicates that up to certain limits of population size, group survival values exist on which evolution can act by selecting a supra-individualistic unit. Continued selection of such units may result in the evolution of increasingly important cooperations. Such selection can readily be a pathway by which higher levels of sociality have arisen from the more primitive types of cooperation we have been discussing.

With Protozoa, for example, there is evidence of the existence of sociality based on a fairly complex system of sexual discriminations such as have been unearthed by Sonneborn, Jennings and others. Sociality in the Protozoa strikes deeper than this. The existence of an optimum population at some intermediate size indicates that there is a tendency toward non-conscious cooperation which was probably the forerunner of all phases of social living.[5]

There is nothing radical in this interpretation of the evidence. All I am doing is to recognize, along with Sewall Wright (1931, 1932), Dobzhansky (1937), A. E. Emerson (1939) and others, that populations are selected as well as individuals. As I said before the Ecological Society of America a year ago, the radical aspect of this matter comes in its implications. One of these is that potential sociality is as inherent in living organisms as are the potentialities of disoperation. This means that social life is not an accident which appears sporadically among a few highly evolved animals. It is rather, as Espinas and Wheeler concluded long ago, a normal and basically a wide-spread phenomenon.

This carries another implication which is particularly pertinent to consider under present world conditions. If we are justified in concluding from evidence such as I have been summarizing that there is an innate tendency toward sociality which is widely distributed among living things, this conclusion bears directly on fundamental

[5] Some such cooperation was a necessary precursor for the evolution of the Metazoa from a protozoan ancestor.

human thought and action. One of the interesting suggestions which develops from these considerations concerns the currently popular pose that science in general and biological science in particular pushes us toward an attitude of human defeatism and general pessimism. Such a tendency is not wholly in accord with the evidence. Disoperative competition is not the only reality in nature. While there are ample grounds, particularly from the short-run view, for being pessimistic about human nature and the biologist's world in general, the facts, or at least a reasonable interpretation of the apparent facts, do not of necessity force us to that position. Rather, for the long-run view at least, an objective survey of all available evidence seems to permit and even to encourage a reasonable and reassuring naturalistic optimism. The biological universe is not wholly unfriendly when a pervasive tendency exists, even among Protozoa, that makes towards non-conscious cooperation. Objective science, if understood, is not as earth-bound as certain religious and secular philosophers teach in season and out, when by the application of the scientific method we can discover evidence not alone of disoperation but also of fundamental tendencies toward cooperation even among the Protozoa.

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SIZE INHERITANCE

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THE inheritance of quantitative characters such as body size was long a stumbling block to students of genetics. It appeared that in such cases the inheritance was intermediate or blending rather than alternative, as in typical Mendelian crosses. The difficulty appeared to have found a satisfactory solution through the investigations of Nilsson-Ehle in Sweden and East and Emerson in America, in what came to be known as the multiple factor hypothesis, which may be stated as follows:

1. When the parents differ in a quantitative character, the F_1 phenotype is commonly intermediate between the respective parents, and not more variable than the more variable parent. The F_2 generation is also intermediate but more variable than F_1, its range in extreme cases extending into that of the parental types, especially if a large population is produced.

2. It is assumed that in such cases the difference between the parents was the resultant of action by several or many different genes located in different chromosomes, that these were devoid of dominance and had a cumulative action. Recombination among such genes was assumed to account for the increased variability of F_2 as compared with F_1 or the parents.

For a long time no particular search was made to determine the number or location in the chromosomes of the assumed quantitatively acting genes. Meanwhile, Castle and Wright devised a formula to show how many gene differences were involved in a particular cross, based on the comparative variability of F_1 and F_2. When it was found that use of this formula resulted in highly improbable numbers of gene differences, it seemed that one or more of the basic assumptions of the multiple factor hypothesis might be unsound. Instead of all genes affecting a quantitative character being devoid of dominance, dominance might be lacking in some cases, partial in others, and complete in still others. Instead of their joint action being cumulative, it might be sometimes cumulative, sometimes conflicting. Location of several size genes in the same chromosome would result in linkage

and so give greater apparent influence to constituents of the group.

No further progress in the solution of the problem seemed possible until genes affecting a size character could be located in particular chromosomes and their relative influence estimated.

With this in mind Castle made a study of a size cross in rabbits in which one parent was several times as large as the other and possessed four genes for coat color, different alleles of which were possessed by the two parents. The problem was to see if any one of these four marked chromosomes contained genes which influenced size and if so to what extent. The result was a complete negative. No indications were found that any combination of these four marked chromosomes favored either larger or smaller size in the back cross generation.

A few years later Green made a similar study in mice in a cross involving three coat color genes, brown, dilution and agouti. He found that brown, which was present in the larger parent race, was associated with larger body size in the back cross generation and concluded that the chromosome which contained the brown allele contained also one or more genes for large body size. This interpretation was questioned by Feldman and by Castle on the ground that the action might be due to the brown gene itself rather than special associated size genes. Castle and his associates have carried out a series of investigations to ascertain which is the correct interpretation and have reached the conclusion, after the study of numerous back crosses involving the black-brown pair of alleles, that the accelerating action on growth must be assigned to the physiological action of the brown gene. This conclusion is supported by an observation of Feldman that in a cross between a small bodied brown race of mice and a larger bodied black race, browns in the back cross population were regularly larger than blacks, whereas the contrary relation should have existed if a size gene linked with brown were involved. Castle has also found that in

a rabbit cross in which brown was introduced in the smaller parent, browns in the back cross population are regularly larger than blacks in both sexes. Castle has also made a rat cross to ascertain the behavior of the brown mutation in a back cross population, with the same qualitative result as in the mouse crosses, browns being larger than blacks, although this conclusion is less certain than in the mouse crosses because of smaller numbers. It seems probable, therefore, that in mammals generally production of brown instead of black pigment in the integument permits greater growth in other body structures. This has been found to be true in mice for two other mutant genes affecting pigmentation, *viz.*, dilution and yellow. Dilution accelerates growth when homozygous to a lesser extent than brown. Yellow increases body size when heterozygous, but is lethal when homozygous. The genes for agouti coat pattern and complete albinism have no detectable influence on body size in mice, but the genes for pink-eye, leaden, pink-eye$_2$, short ear and dwarf have retarding effects on growth increasing in the order named.

It appears accordingly that the mutant genes, which we have been able to identify as influencing body size, are not all alike either qualitatively or quantitatively in their influence on body size. Some increase body size, some diminish it and others are neutral. The best estimate which we can form of the influence of each gene on body size in back cross populations, is shown in Table 1.

PERCENTAGE CHANGE IN BODY SIZE EFFECTED BY CERTAIN GENES IN MICE

Gene	Weight*	Body-length	Tail-length
bb	+ 4.27	+1.51	+1.30
dd	+ 2.10	+0.90	+2.64
A^{ya} ♂♂	+33.00	+2.60	+1.50
A^{ya} ♀♀	+62.00	+4.90	+0.20
aa	0	0	0
cc	0	0	0
pp	− 1.01	−0.14	−0.72
ln ln	− 3.64	−0.61	−2.94
p_2 p_2	− 5.90	−2.46	−3.29
se se	− 4.42	−0.72	−0.78
dw dw	−75.00†		

* Naturally the percentage effect on weight (a three dimensional character) is uniformly greater than that on either body-length or tail-length (one dimensional characters), but qualitatively the change is the same by all three criteria of body size.

† Estimate by Snell.

To discover the effect of the size genes of mice when they act in combination has been investigated as yet only in the case of the brown gene. The apparent effects of interaction between brown and other size genes of mice are shown in Table 2. Brown and dilution in combination

TABLE 2

PERCENTAGE CHANGE IN BODY SIZE EFFECTED BY INTERACTION OF THE BROWN GENE WITH OTHER SIZE GENES

Gene	Weight	Body-length	Tail-length
bb dd	+5.81	+2.70	+3.89
bb Ln ln	+1.07	+0.71	+0.55
Bb ln ln	−3.64	−0.61	−2.94
bb ln ln	−5.47	−1.00	−3.42
bb P_2p_2	−0.70	−0.36	−0.07
Bb p_2p_2	−5.53	−1.89	−4.04
bb p_2p_2	−5.90	−2.46	−3.29

(bb dd) increase body size beyond that effected by either gene acting alone. Compare Table 1. This combination appears to be favorable. But where brown is associated in like manner with the unfavorable genes, leaden and pink-eye$_2$, its favorable action is reversed so that the combined action of brown and leaden or brown and pink-eye$_2$ is to reduce body size more than either leaden or pink-eye$_2$ would by itself reduce body size.

When brown is homozygous and leaden heterozygous (bb Ln ln) the accelerating effect of brown is greatly diminished but not cancelled. When leaden is homozygous and brown heterozygous (Bb ln ln) size is diminished less than when both are homozygous (bb ln ln).

When homozygous pink-eye$_2$ is associated with brown, either heterozygous or homozygous, size is diminished more than in the corresponding combination with leaden. In general, we may say, brown ceases to be a beneficial gene, when associated in any combination with genes ln and p_2, since the resultant action is less favorable than that of the respective genes acting apart. Also the gene p_2 is more effective than ln in reducing body size either separately or in combination with gene b, the accelerating effect of which it completely reverses.

One point of uncertainty regarding size inheritance has long been the relative influence of the father and the

mother on the size of offspring. If the body size of offspring is determined exclusively by the action of genes contained in the gametes, we should expect the influence of egg and sperm to be as exactly equivalent as the chromosome content of egg and sperm, respectively, which are identical except as regards the X and the Y chromosomes in offspring of the heterozygous sex (males in mammals, females in birds). On the contrary it has been found in several well established cases that in reciprocal crosses between breeds, races, or species of unlike body size, the mother has greater influence than the father on the body size of the offspring, since when the mother is of the larger bodied race the offspring are larger than when she is of the smaller bodied race. A well known example is found in reciprocal crosses between horse and ass, the mule having a mare as mother being a larger animal than the hinny, the mother of which is a donkey. Castle found the same to be true in reciprocal crosses between races of rabbits differing in body size, and a still larger difference has been found by von Ubisch and Mello in reciprocal crosses between *Cavia rufescens* and the domestic guinea pig. In this case the *C. rufescens* parent is about half as large as the guinea pig, and the young in each cross resemble the mother's race in body size much more closely than the father. The result is undoubtedly due in part to differences in conditions of gestation in large and small mothers respectively. In a large female mammal the uterus is larger and the nourishment supplied from it to the foetus undoubtedly more abundant. In an animal with a prolonged gestational period this influence will be greater than in one in which the young are born in a less advanced stage of development. For example, gestation is more than twice as long in the guinea pig as in the rabbit and the young guinea pig is born with eyes open, fully furred and able to feed independently of the mother, whereas the new born rabbit is naked, blind and unable to feed itself. And the difference between reciprocally produced young is much greater in guinea pig crosses than in

rabbit crosses. But gestational differences will not account wholly for the greater size of young having a large sized mother. This is shown by the existence of a similar difference in size between reciprocally produced hybrid young obtained in crosses of amphibian species of different body size. In such cases there is no gestational influence, the development of the embryo taking place wholly outside the body of the mother subsequent to fertilization of the egg, yet here also the mother of the larger species has larger sized offspring, as shown by the observations of Hamburger and Pariser. We may reasonably suppose that in such cases, a reserve of food materials has been stored in the cytoplasm of the egg before its fertilization, and that such reserves are larger in a large bodied species than in one of smaller body size. In mammals the food reserve stored in the egg prior to fertilization is less than in amphibia, and this is supplemented by nourishment supplied through the placenta during gestation.

The superior influence of the mother on the body size of offspring may, if one likes, be regarded as maternal inheritance, but is perhaps better regarded as a developmental rather than a genetic influence. For the germ plasm is not permanently made different by the maternal element. It will be handed on to later generations of progeny only through the daughters, since the sons produced in the two reciprocal crosses, though differing in body size, do not hand this difference on to their progeny. It is purely a somatic difference not affecting the potentialities of the sperm produced by the male, where only chromosomal influences can be detected.

That chromosomal constitution of the gametes also influences the body size of offspring is shown by observations of Castle that the offspring of a female rabbit of a large bodied race are larger when the sire is of a large bodied race than when he is of a small bodied race. This shows that the sperm as well as the egg exerts an influence on the body size of offspring, an influence which in all probability is chromosomal in origin since the cyto-

plasmic constituent of the sperm is so small as to be negligible.

Another interesting question about size inheritance is concerned with the method by which size genes influence body size, whether by influencing the size of the body as a whole or by influencing the size of particular parts of the body. In the early days of Mendelism it was supposed that each organ or structure of the body had its special determining genes, and if these by mutation were lost, the organ would disappear. This view was reflected in the prevailing terminology of "unit characters" and "unit factors." In accordance with this view it was held by one investigator (though on quite inadequate evidence) that a cross between large bodied and small bodied races of rabbits resulted in later generations in disharmony of size of parts, such as long legs on short bodies, or *vice versa*. Applying the idea to man, crosses between the different races of man were discouraged on the ground that they would inevitably result in physical and mental states of unbalance or disharmony, no consideration being given to the idea that the disturbing effects of racial crossing might be due to social rather than physical inheritance.

Later it was found that a single gene may influence simultaneously various parts or processes in the body. Thus in mice a gene which has its most obvious effect in shortening the ear and so is known as the short ear gene, has effects also on the length of the vertebral column and the tail, as well as on total weight of the body. Also a gene which has its immediate effect on the size and secretory activity of the pituitary gland, through such activity slows up the process of growth, resulting in a dwarfed body which never attains reproductive maturity.

By a comparative study of the embryology of large race and small race rabbits and of their hybrids, it was found by Castle and Gregory that the general size of the body is determined in the fertilized egg very early, probably not later than fertilization, for in early cleavage stages it is

found that the eggs of large race rabbits develop more rapidly than those of small race rabbits, new cells being formed faster, so that the blastocyst is larger and a bigger embryo arises from it. The more rapid rate of growth persists throughout both the prenatal and the postnatal periods. Thus the large race rabbit is not only larger at birth but it continues to grow faster and longer after birth. Its growth trajectory, so to speak, is higher, the charge behind the projectile being greater so that it rises higher and goes farther before it comes to rest.

Notwithstanding the fact that general body size is early determined in the fertilized egg and thus influences all parts of the body simultaneously and equally, it is true that there are also genes the principal effect of which is seen in particular organs, parts or processes. Such are the genes which affect hair length, ear length and tail length in various mammals, which to a greater or less extent are independent of general body size. MacDowell and Wright have shown that sex is a character which affects relative length of hind legs and width of the skull in rabbits, in which respects males exceed females. Such effects are an indirect consequence of the action of genes carried in the Y-chromosome (or of absence from the Y-chromosome of genes carried in the X-chromosome). Castle, *et al.,* have shown that in mice the gene for dilution which has a less effect on general body size than the gene for brown, nevertheless has a greater effect on tail length.

Wright has concluded, from a study by his ingenious method of path coefficients of data on the size of various parts of the skeleton of rabbits recorded by MacDowell and by Castle, that the inheritance of body size is chiefly *general, i.e.,* by differences in developmental rate (Castle and Gregory) affecting all parts of the body. To a lesser extent influences (genetic or developmental) change in the same direction groups of organs such as front and hind legs in mammals, or wings and legs in birds. And finally individual parts (head, tail, etc.) may vary inde-

pendently of the size of the body as a whole. In general, Wright finds that these several aspects of variance (genetic or developmental) rank about as follows in rabbits: general (affecting size of the body as a whole) about 65 per cent.; group (affecting head or limbs independently of the entire body) about 15 per cent.; special (affecting particular organs or parts) about 20 per cent. In other groups of mammals these relations would certainly be very different. Thus in the giraffe group factors would be very important in causing simultaneous elongation of neck and front legs, whereas in the kangaroo important group factors would cause elongation of hind legs and tail out of all proportion to general body size or to the size of the front legs.

SUMMARY

1. The multiple factor hypothesis in its original and simplest form is an inadequate explanation of the facts known to us concerning the inheritance of body size in mammals. It is correct in so far as it postulates an influence on body size exerted by many independent genes. It is incorrect if it assumes that the influences of such genes are equal or uniformly in one and the same direction.

2. Experimental studies of size crosses, chiefly in mice, have shown that a majority of the common mutant genes have an influence also on body size. Some of these increase body size, others decrease it, while still others have no observable influence on body size.

3. The first discovered and most conspicuous example of a color gene which influences body size is that of the brown mutation, which increases body size in mice, rats and rabbits. The influence of blue dilution in mice is of the same nature but less in amount. Brown and dilution in combination have a greater accelerating effect on body growth than either acting alone.

4. The interaction of brown with other color genes is peculiar. Genes leaden and pink-eye$_2$, which retard body growth, act together or in combination with brown so as to retard growth more than either does by itself. Thus

the stimulating action of brown is reversed when it is associated with leaden or pink-eye$_2$, and the combination of these two is more retarding than either by itself.

5. In reciprocal size crosses in animals, the mother has greater influence than the father on the body size of the offspring. This in mammals is primarily a gestational influence, but in part also an influence of the cytoplasm of the egg, since a similar difference is found among amphibia in which group a gestational influence is absent. This influence can not be regarded as genetic except in so far as the genotype of the mother influences the cytoplasmic constitution of her eggs.

6. Concerning the mode of action of size genes, experimental size crosses studied statistically by Wright lead to the conclusion that size genes act chiefly by influencing developmental rate of the embryo (Castle and Gregory) since general body size is thus affected. To a lesser extent gene action affects groups of organs or parts independently of general body size, and to some extent also special organs or parts are affected independently of general or group gene action.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

Further Studies on the Opalinid Ciliate Infusorians and Their Hosts. By MAYNARD M. METCALF. Proc. U. S. Nat. Mus., 87, 1940: 465–634, figs. 21–157.

THE author states that this paper should be read in connection with "The Opalinid Ciliate Infusorians" (*Bull. U. S. Nat. Mus.*, 120, 1923), of which it is a revision and a second part. "About 30 new species and subspecies are described; species described by others since 1923 are considered and illustrations and measurements copied; the taxonomy of the family is reviewed, as well as the data and hypotheses as to geographic distribution; and former reviews of the literature (Metcalf, 1909 and 1923a) are brought to date. Thus Bulletin 120 and the present paper together cover the family Opalinidae as now known."

I note two omissions from the bibliography. One is my own criticism (Dunn, AMER. NAT., 59, 1935: 370–75) of the zoogeographic and amphibian portion of Metcalf's former paper, and the other is Noble's critique (AMER. NAT., 59, 1935: 265–71) of the same section. These criticisms are not answered in the text.

The opalinid parasites of the genera *Protoopalina, Zellerella, Cepedea* and *Opalina* are now known from termites (*Opalina* in India), fish (*Protoopalina* in a marine fish from the Mediterranean and *Zellerella* in a fresh-water catfish from the Paraguay River), salamanders (*Protoopalina* in *Ambystoma tigrinum* in the United States and in *Triturus vulgaris* in Europe;

Opalina in *Triturus alpestris* in Europe), frogs of many families, lizards (*Protoopalina* in *Varanus niloticus* in Africa), and snakes (*Zellerella* in *Xenodon merremii* and in *Liophis jaegeri* in Brazil).

All genera of frogs of which 10 or more species have been found parasitized (*Bufo,* 44; *Rana,* 37; *Hyla,* 28) are infected by all 4 of the genera of Opalinidae, and so are *Scaphiopus* with 6 parasitized species and *Microhyla* with 5. *Polypedates* and *Eleutherodactylus,* with 6 parasitized species, maintain 3 genera of these parasites. *Leptodactylus* and *Hylorana* with 8 parasitized species, and *Bombina* and *Paludicola* with 4, each harbor 2 genera of parasites. No genus of frog of which more than 3 species have been found parasitized maintains less than 2 genera of the opalinids.

Protoopalina intestinalis is said to parasitize 9 species of frogs (representing 7 genera and 6 families) and a salamander. *Opalina obtrigonoidea* is said to parasitize 13 species of frogs, belonging to 5 genera and 4 families. *Opalina ranarum* is said to parasitize a salamander, and 8 frogs of 4 genera and 3 families.

The manner of transmission of parasites is said to be from tadpole to tadpole (via water), and *Pipa* and its allies, as well as the New Zealand *Liopelma,* are said to lack these parasites because of the absence of a free-living tadpole stage. Six species of *Eleutherodactylus* (a genus which lays eggs on land, and which has no tadpole stage at all) are said to be infected by *Protoopalina, Zellerella* and *Cepedea.*

A broad picture of opalinid distribution is: the two more specialized genera, *Cepedea* and *Opalina,* are absent from the Australian region (which has only the primitive *Protoopalina* and *Zellerella*); "Opalinae latae" are absent from South America (but the more specialized "Opalinae angustae" are present there); the primitive *Zellerella* is largely confined to Southern regions; the specialized "Opalinae angustae" are the only opalinids found in eastern North America; Madagascar has only the two more specialized genera.

In many groups of animals the primitive forms are to be found in the southern areas, and the modern forms in northern areas. This sort of distribution is usually held to indicate northern origin, and I am inclined to think it indicated for the Opalinidae.

The distribution of opalinid genera does not agree very well with the distribution of frog groups. For instance, Eastern North America has so far produced only the genus *Opalina,* and indeed only the section "Opalinae angustae," but this area is inhabited by 6 families of frogs, including 8 genera and 30 species.

The portion of this paper (and that of the previous one) which is concerned with facts as to opalinids is a distinct contribution and is not criticized. There is, however, in both papers, an elaborate attempt to infer dates and areas of origin and routes of dispersal both of opalinids and of frog groups; an attempt which involves as additional inference great modifications of the surface features of the earth. These sections, in the present paper, are almost identical with those in the previous one. That *Opalina* is in South America and that *Zellerella* is in China and South Africa, reported now in this paper, is not allowed to affect the older inferences. The principle of Occam's razor "that entities are not to be unnecessarily multiplied" is not followed. Many nonexistent land areas are inferred. It is not necessary to infer these if one infers extinction of frogs and opalinids. The land areas are inference pure and simple; extinction is not all inference, as we have remains of extinct forms.

The use of data from parasitology to indicate relationships of hosts has recently been reviewed critically by Baylis ("Evolution," 1938: 266), and Zuckermann ("Functional Affinities of Man, Monkeys and Apes," 1933) has summarized the situation in Primates. Both of these authors are very sceptical as to coincidence in phylogeny of host and parasite. This is the only legitimate and true use of the "host-parasite method" but it is not used by Metcalf, as the wide variance between

classification of opalinids and that of frogs renders it manifestly impossible (*e.g., Protoopalina,* subgeneric group 1, infects a teleost fish and 5 families of frogs).

The use of data from parasitology to show geographical and geological relationships of hosts is less legitimate and less necessary. Even if the classification and distribution of host group and parasite group agree closely the parasites tell us little that we can not infer from the hosts. Johnstone (Rep. Austral. Assoc. Adv. Sci., 1914: 72) states that trematode parasites of Australian frogs have Palearctic affinities, a view entirely opposed to Metcalf. The same author (*Jour. and Proc. Roy. Soc. New South Wales,* 1916) says that cestodes and trematodes of Australian and South American marsupials show no close relationship, which disagrees with Metcalf's views concerning opalinids and frogs of these regions.

Many of Metcalf's statements concerning present frog distribution are incorrect, and the classification of frogs that he uses is antiquated and erroneous. As even the modern classification contains many uncertainties and defects no great confidence can be placed in any scheme of origin and dispersal, and none in one based on such erratic parasitism as the opalinids display, or in one which calls for so many hypothetical land areas.

Many of the geological and geographical inferences are misleading. For example, it is not necessary to invent a seaway between Brazil and Patagonia to account for the absence of hylid tree-frogs from the Patagonian desert, but Metcalf does.

Extinction is not "a wholly improbable hypothesis" to herpetologists, all of whom are sadly aware of the proven extinction of whole orders of amphibians and reptiles. Even Metcalf sometimes admits the possibility of extinction. Thus *Liopelma* of New Zealand (which has no parasites) is supposed to have been formerly in Australia and to have become extinct there, but to have left its parasites to other frogs. His statement (p. 601) concerning microhylid frogs, "former spread across the Malayan

islands with later extermination in most of these," is another admission of possible extinction, but an amusingly gratuitous and erroneous one as the animals are present and abundant in all the known islands. I have myself taken examples in Java, Lombok and Komodo. Metcalf's statement that "there are no eastern Asian species" of this group is incorrect, as they occur as far north as Peking and are abundant in east China and the Malay Peninsula.

Numerous other erroneous statements by Metcalf regarding the taxonomy and distribution of frogs could be cited, to illustrate the criticisms here offered.

EMMETT REID DUNN

Protozoa in Biological Research. Edited by GARY N. CALKINS and FRANCIS M. SUMMERS. New York: Columbia University Press, 1941: i–xli, 1–1148. $10.00.

THE editors of this large compendium explain that it is not intended as a substitute for a text-book on Protozoa, but rather a means to stimulate further research on the one-celled animals. The authors—leading American protozoologists—have treated the results of research in the various phases of the subject, on which they are outstandingly competent to write. Emphasis on general biological significance has been an aim. Geneticists and students of evolution will be particularly concerned with "Fertilization in Protozoa," by John P. Turner; "Endomixis," by Lorande Loss Woodruff; "Sexuality in Unicellular Organisms," by T. M. Sonneborn, and, particularly, in "Inheritance in Protozoa," by H. S. Jennings. In this chapter, cytoplasmic inheritance is particularly emphasized, and a test is suggested to determine whether such inheritance is alone involved in the inheritance, for hundreds of generations, of modifications due to acclimatization.

Man on His Nature. By CHARLES SHERRINGTON. New York: Macmillan Co., 1941: 8 + 1–413, pls. 1–7, 5 figs. $3.75.

AN outstanding English physiologist, Nobel Prize winner for Medicine in 1932, Charles Sherrington expounds

at length on the reason, purpose and future of human life. He obviously attempts to present the current conceptions of man's nature, as these have been molded by scientific research, and to contrast the present interpretations with the tenets of ancient, medieval and modern philosophers. The evolution of man's thoughts regarding himself is particularly indicated by quoting Jean Fernel, who is pictured as a sixteenth-century leader of outstanding influence in the fields of medicine, of physiology in its nascent stage, and of natural philosophy in general. Fernel's philosophy—regarded as a reflection of better thinking of his age—was spoken through the mouth of the "Eudoxus" of his *Dialogue;* Sherrington's views, exemplifying those of the modern scientific world, are presented through the de-personified diction of science. It seems to the reviewer that the most enlightening contribution Sherrington has here made is to the history of human thought regarding *Homo sapiens,* and that was apparently the author's intention. There is no attempt to monograph man's nature; no original philosophy of man is introduced; no startling theories advocated. However, enough pertinent data from medicine, physiology, psychology and other sciences is introduced (stripped of technicalities) to render it obvious that we read the historical and philosophical reflections of a great mind, skilled in the methods of experiment, experienced in the requirement of verification, unaccustomed to the plucking of convictions out of thin air.

Biology of the Laboratory Mouse. By the staff of the Roscoe B. Jackson Memorial Laboratory; with a chapter on the infectious diseases of mice by J. H. Dingle. Edited by GEORGE D. SNELL. Philadelphia: The Blakiston Company, 1941: i–ix, 1–497, figs. 1–172. $7.00.

INCLUDED are excellent accounts of the reproduction, embryology, histology and genetics of the house mouse. Considerable attention is given to neoplasms and their genetics. Chapters especially valuable to the laboratory worker deal with parasites and infectious diseases. The

book is a valuable reference work for any one engaged in laboratory studies of house mice.

LEE R. DICE

NOTICES OF NEW BOOKS

The flood of new literature of interest to general biologists swamps any effort to review in detail more than a few selected items. Other books will be listed with a few explanatory remarks, in the following fashion.

Butterflies A Handbook of the Butterflies of the United States, Complete for the Region North of the Potomac and Ohio Rivers and East of the Dakotas. By RALPH W. MACY and HAROLD H. SHEPARD. Minneapolis: University of Minnesota Press, 1941: i–vii, 1–247, col. pls. 1–7, 38 figs. $3.50.—This manual is accompanied by an introduction treating various subjects of general interest, particularly emphasizing questions of zoogeography. A feature of the text is the treatment as intergrading subspecies of several forms commonly regarded as species.

A Laboratory Manual for Histology. By James Forbes. New York: Fordham Univ. Press, 1941: 1–74, figs. 1–3. $1.25.

L. M. B. C. Memoirs on Typical British Marine Plants and Animals. XXXIII. Pomatoceros Sabella and Amphitrite. University Press of Liverpool, 1940: 1–88, figs. 1–7, pls. 1–11. 10/6.—Another of the valuable morphological monographs of type animals, issued by the Liverpool Marine Biology Committee.

The Boy's Book of Insects Interesting Facts about the Lives and Habits of the Common Insects together with Simple Instructions for Collecting, Rearing and Studying Them. New York: E. P. Dutton & Co., 1939: 1–237, figs. 1–24, 31 pls. $2.00.

Comparative Chordate Anatomy A Laboratory Text. By Clair A. Hannum. Stanford University, California: Stanford Univ. Press, 1941: i–vii, 1–211, 4 figs. $2.00 (paper-bound).—One of the almost innumerable new texts in comparative anatomy, good like many others. It retains the type-study method of presentation.

Studies of Neotropical Colubrinae. VIII. A Revision of the Genus *Dryadophis* Stuart, 1939. Misc. Publ. Mus. Zool. Univ. Mich., 49, 1941: 1–106, figs. 1–13, maps 1–4, pls. 1–4. $1.15.—

The analysis of variation in this snake genus, the indication that some of the young have a reduced number of scales (a "supposed lethal character"), the study of sexual dimorphism, and the discussion of clines and evolutionary trends, will prove of interest to students of speciation.

THESES IN MICROFILM

The publication of doctorate theses is becoming increasingly difficult. To meet this situation the practice of issuing theses in microfilm has recently been established. University Microfilms, Ann Arbor, Michigan, is now reproducing theses at a uniform cost of $15.00, and is supplying copies at 1¼ cent per page on microfilm, or 6 cents per page on paper enlargements. Theses available in this new form of publication are listed in "Microfilm Abstracts. A Collection of Abstracts of Doctoral Dissertations Which Are Available in Complete Form on Microfilm." Several theses in zoology are among those which have thus been issued and abstracted.

SHORTER ARTICLES AND DISCUSSION

"MISTY" A NEW COAT COLOR DILUTION IN THE MOUSE, *MUS MUSCULUS*

An exceptionally light-colored male mouse was found[1] in the Jackson Laboratory dilute brown strain of mice (JAX dba, *ddbbaa*). The exceptional male also possessed a white tail tip and a unilateral rectangular-shaped white belly spot. White spotting or marked variations in color are not characteristic of the parental stock.

The variation is obviously distinct from other dark eye mutations causing general color dilution in the mouse. Three locations for these have been known for a number of years, the albino allels c^e and c^{ch}, leaden *lnln* and blue dilution *dd*. Crosses between the exceptional male and both the albino allels and leaden gave intense colored individuals in the first generation. That the variation was not an allel of *d* was shown by crossing the light male to *DDbbaa* individuals and backcrossing the F_1 females. Four classes of young were obtained, new-light like the sire, dilute brown, brown and also one with only a slight dilution from full color brown. Further study of the latter class showed that they could eventually be bred true and were undoubtedly a new mutation now separate from the *dd* of the JAX dba stock. The mutant gene was then isolated on a black background and again produced a slight dilution of a degree not heretofore observed in mice. One of the few single alphabetical symbols not now used in mice *m* (misty) was used to designate this mutant gene. It is still possible that it will be allelomorphic to some known gene and the designation will need to be changed.

mmbbaa and *mmBBaa* mice are more uniform and more intense in color than *lnlnbbaa, lnlnBBaa* or *ddbbaa* and *ddBBaa* mice. Microscopic examination of the hairs shows *mm* individuals to have much more cortical pigment and probably slightly more medullary pigment than *dd* or *lnln* mice on both *bbaa* and *BBaa* backgrounds. On microscopic examination *dd* and *lnln* mice were very similar except for more pigment clumping in the *lnln* individuals which were examined. Further study is needed to show whether this is a characteristic of modifiers or the main

[1] The exceptional mouse was first noticed and saved in the author's stock by Allen Salisbury, assistant at the Roscoe B. Jackson Memorial Laboratory.

gene. *mm* seems to allow more pigment in extremities such as the hair tip, ears and tail than either *lnln* or *dd*.

The above mutation is the first general coat color variation noted and published on in the dilute brown strain since it was originated by Dr. Little in 1909. The strain has been produced in large numbers during the many years of its existence, and used extensively in genetic and physiological studies in many countries.

The particular subline in which the mutation occurred has not been crossed any wider within the strain than brother-sister for the last twenty-six generations. It has been possible to continue the inbreeding and at the same time produce a new true breeding misty-dilute, *i.e.* (*ddmmbbaa*) branch of the stock. The white tail tip and an occasional belly spot is a characteristic of the new branch but not in the dilute *ddMmbbaa* segregates which appeared during the start of the new sub-strain. It is possible that the spots may prove to be a part of the expression of the gene *m* when present in a homozygous condition and with certain other combinations of genes.

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OBSERVATIONS ON CATHAEMASIA RETICULATA, A TREMATODE FROM THE BELTED KINGFISHER

THROUGH the generosity of Assistant Professor Merrill Wood, an ornithologist of the department, three specimens of *Cathaemasia reticulata*, a trematode of the family Echinostomatidae, from the intestine of the belted kingfisher (*Megaceryle alycon*), were received for identification and study. Unfortunately, the anterior end of one worm was missing and the remainder did not section well. The other specimens were stained with Delafield's haematoxylin and mounted for study. Slight pressure between slides was applied.

Since the initial description by Wright (1879) is lacking in detail and considerable variation exists with that given by Harwood (1936), some additional notes on the species were considered of interest and supplemental to the previous diagnosis.

DISCUSSION

The correct location appears to be the intestine of the host. Dr. E. W. Price, of the Zoological Division of the Bureau of Ani-

TABLE I

COMPARATIVE DESCRIPTIONS

	After Wright	After Harwood	After the Author
Specimens studied	2	1	2
Shape	ovate	oval	slightly oval
Color	——	——	centre light gray, with dark gray border
Anterior end	neck-like	constricted	slightly cone-like
Length	14.0 mm	9.2 mm	17 to 18 mm
Width	8.0 mm	3.4 mm	6 to 8 mm
Spines	25 μ	10 μ	15 to 20 μ
Oral sucker	subterminal 0.9 mm	subterminal 0.54 by 0.64 mm	subterminal 0.85 mm
Mouth	circular	——	0.6 mm
Pharynx	0.48 mm wide, oval	0.35 long by 0.3 mm wide	0.39 wide by 0.45 to 0.54 mm long
Esophagus	——	0.24 mm long	0.37 long, to outer intestinal arc, by 0.3 mm wide
Caeca	unbranched	unbranched terminate near posterior end	same, left slightly shorter, right 0.6 mm from posterior end
Seminal vesicle	——	piriform, 0.54 mm long by 0.33 wide	vase-shaped 0.78 mm long by 0.73 wide
Genital pore	just anterior to ventral sucker	——	visible
Genital atrium	——	——	oval, 0.3 by 0.45 mm
Ventral sucker	1.3 mm	0.7 mm in diameter	oval, 1.2 to 1.3 mm long by 1.0 to 1.2 mm wide
Cirrus sac	——	——	not seen
Cirrus	?	retracted	retracted, indistinct
Uterus	dorsal to ventral sucker	from ovary to genital pore	0.3 mm wide, ovary to genital pore between caeca
Vagina	——	——	not observed
Egg	110 by 65 μ	110 by 70 μ, yellow	120 by 75 μ, yellow
Ovary	——	0.54 mm long by 0.29 wide	0.9 mm long by 0.45 wide
Mehlis' glands	——	size of ovary	size of ovary
Ootype	?	——	observed
Laurer's canal	——	present; opening shown	observed, except external opening
Seminal receptacle	——	not present	not present
Vasa efferentia	——	right and left	right and left
Vas deferens	——	not shown	not observed
Vitellaria	racemose	follicular	follicular
Vitelline ducts	transverse and common	prominent transverse and common shown	prominent transverse, longitudinal also common
Testes	branched	prominent, posterior larger	posterior the larger
Excretory vesicle	——	Y shaped, short	Y shaped, longer
Excretory pore	——	not drawn	terminal

mal Industry, U. S. Department of Agriculture, informs me that others have recently requested information on specimens of this fluke from the kingfisher so it is apparently being occasionally found. Further data on these specimens have not been obtained. Wright (1879) illustrates the worm as very oval at the posterior end and tapering rapidly toward the anterior end. The ones studied are much like the one described by Harwood (1936),

having the sides nearly parallel for two thirds the length. The cone-like anterior end beginning at the ventral sucker is less noticeable. A considerable variation in the length of the worms at various ages is to be expected. The spines are rather indistinct except along the border. The one longer caecum is not mentioned by the above authors. The walls of the uterus do not show through the major length of the tube. The eggs are light yellow and are very numerous. A prepharynx is absent. In the drawing only the vitellaria that are ventral to the caeca are shown and those median to them. The latter are continuous with those dorsal to the caeca and the entire areas between the caeca and lateral yolk ducts are filled. There is an inconsistency between the location of the vasa efferentia as shown by Harwood in his drawing and the description. The one from the anterior testis goes to the left and the one from the posterior testis to the right side in the anterior third of the worm. The posterior testis is the larger and the branches do not anastomose as stated by Wright and refuted by Harwood. From the U. S. National Museum, Harwood's specimen and two of Edney's from Tennessee were examined. One of the latter is as large as the author's specimens. In it, the left vas efferens arises from the center of the stem of the anterior testis and the vasa efferentia are traceable to the posterior end of the seminal vesicle which they seem to enter singly. An oviduct leading to the ootype can be seen. These are not shown in the author's illustration. In Edney's excellent larger specimen the eggs measure 92 by 126.5 micra, exceeding those in the author's and the cone-like end is more prominent. The fine branches of the excretory system are visible under the low and high powers of the compound microscope, including flame cells under the latter. Harwood does not show the excretory vesicle in his illustration. The spines are more abundant at the anterior end. Their lengths vary some and the tips are recurved.

Fuhrman (1928) places the genus in the family Cathaemasidae. Travassos (1939) placed this trematode in the genus *Pulchrosoma.*

Synonyms: Distomum reticulatum Wright, 1879. *Distomum* (*Brachylamus*) *reticulatum* Stossich, 1892, *Fasciola reticulata* Looss, 1899, *Cathaemasia reticulata* Harwood, 1936, *Pulchrosoma reticulata* Travassos, 1939.

Other Species: C. hians (Rudolphi); *C. spectabilis* (Odhner); *C. famelica* (Odhner).

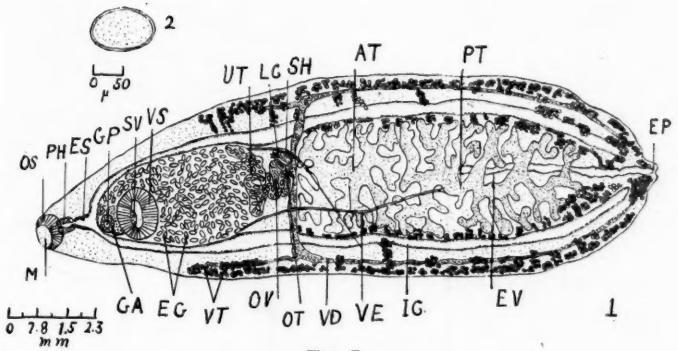

FIG. I

(1). Ventral view of specimen. Abbreviations: M, Mouth; OS, Oral Sucker; PH, Pharynx; ES, Esophagus; GP, Genital Pore; GA Genital Atrium; SV, Seminal Vesicle; VS, Ventral Sucker; EG, Eggs; VT, Vitellaria; UT, Uterus; OV, Ovary; LC, Laurer's Canal; SH, Mehlis' Gland; OT, Ootype; VD, Vitelline Duct; VE Vasa Efferentia; AT, Anterior Testis; PT, Posterior Testis; IC, Intestinal Caecum; EV, Excretory Vesicle; EP, Excretory Pore.
(2). Egg.

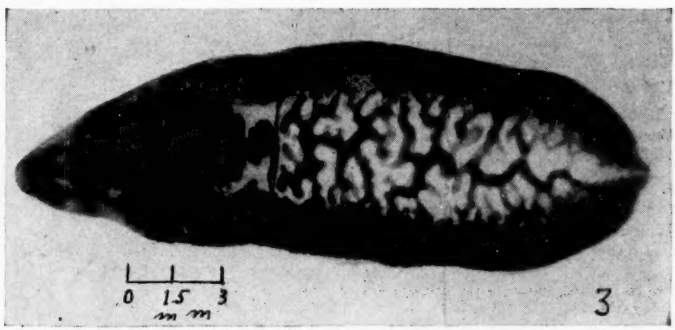

FIG. II

A key is given by Harwood for the species. There is a considerable resemblance between this genus and *Parafasciolopsis*, Ejsmont, 1932. *Parafasciolopsis fasciolaemorpha* was described from European elk by Ejsmont (1932). The testes are somewhat similar in the two genera. Ullrich (1937) gives further details concerning *Parafasciolopsis* (Family Fasciolidae). The transfer to the genus *Pulchrosoma* Travassos, 1916, seems inopportune for members of *Cathaemasia*, Looss, 1899, and the validity of the former genus is questioned by some. *Chataemasia*, by Travassos, appears to be an oversight in copying in his 1939 paper.

Host: Belted Kingfisher (*Megacercyle alycon*).

Location: Intestine.

Distribution: Pennsylvania.

Specimen: U. S. Nat'l. Museum Cat. No. 36768.

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